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MECHANICAL MODIFICATION OF DOUGH TO MAKE IT POSSIBLE TO BAKE BREAD WITH ONLY THE FERMENTATION IN THE PAN[1]

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The use of yeast in bread making antedates written history and the making of light bread by the use of yeast is one of the great discoveries of mankind. For growth the yeast plant needs some soluble proteins, carbohydrates, and mineral substances, which are all present in dough made in the customary way. The rate of yeast activity is most dependent on a plentiful supply of soluble carbohydrates, hence these are usually supplied by the addition of sugar in some form. The main by-products of growth are carbon dioxide and alcohol, which are produced at the expense of flour material and added sugar. Some authorities state that from 2 to 4 per cent of the flour is consumed by the yeast in the ordinary process of dough fermentation. It follows, therefore, that any process of bread making which shortens the time necessary for fermentation will result in a considerable saving of material.

The main purpose of fermenting dough is to produce a porous structure. As soon as gas begins to form, the gluten meshes are stretched. The water film on the gluten mesh network forms a membrane impermeable to such an extent that gas forms faster than it escapes, giving the light, spongy texture. When such fermented dough is placed in the oven the heat coagulates or "sets" the structure and we obtain porous bread.

It is a matter of common knowledge that the growth of yeast in dough results in a modification of gluten texture. Gluten from fermented dough has lost some of its toughness, and it is more easily stretched. If straight dough is placed at once in the pan

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as soon as mixed, and then baked when it has reached the required volume, the texture will be very different from that of a loaf baked from dough which is allowed to ferment to a large volume, then worked down and allowed to rise again, this repeated one or more times. This modification of the gluten may be partly brought about by the acidity developed during fermentation, partly by the activity of proteolytic enzymes, and it may also be due in part to the mechanical stretching caused by the expanding gas bubbles.

That gluten can to a certain extent be modified by mechanical action, is known to bakers who have used different dough mixers. The rate of speed in a mixer is of particular importance. When the dough begins to form it has a more or less granular appearance. As the mixing proceeds this gives way to a smooth, silky texture, and the elastic limit is greatly increased, which means greater gas-retaining capacity or, in common terms, it is developed.

That mechanical action on dough may be made to take the place of the usual fermentation previous to panning has been demonstrated in the experiments reported in this paper. Bread has been produced which was just as light, had as good texture as bread baked in the ordinary way, and even better color. This has been accomplished by means of a new dough mixer which acts on the dough in a pack-squeeze-pull-tear fashion. The pack-and-squeeze action causes the gluten particles to adhere to each other, forming strands; the pulling elongates these strands and effects thoro intermingling of the starch and gluten particles with the yeast and other ingredients in the dough, the result being a porous network permeating the entire dough mass. The tear action partly breaks down the gluten colloid complexes and so causes a modification of dough quality.[2]

Description of Dough Mixer

The machine as first used is shown in Figure 1. A few minor changes have been made which make it more efficient. The mixer is made for a one-pound loaf. The bowl which holds the dough is made of tinned copper and the mixing is accomplished by four pins having a double or epicycloidal motion, which may also be described as planetary. These moving pins alternately straddle and hurdle three pins fixed to the bottom of the bowl. It is the motion of these pins with reference to each other that produces the pack-squeeze-pull-tear action and brings about the modification.

2 The authors desire to express their indebtedness to C. E. Pearce, professor of Machine Design and to W. W. Carlson, professor of Shop Practice, for help in designing and constructing this dough mixer.

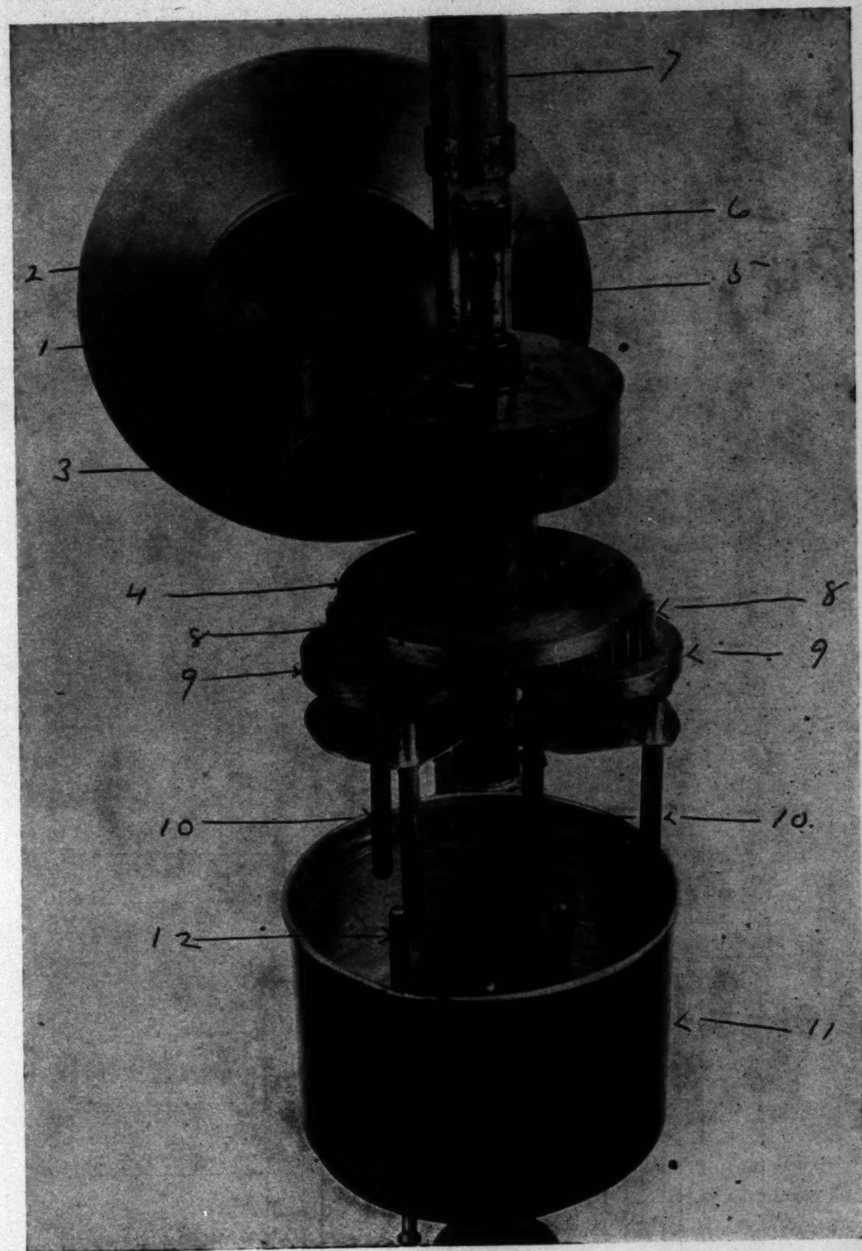
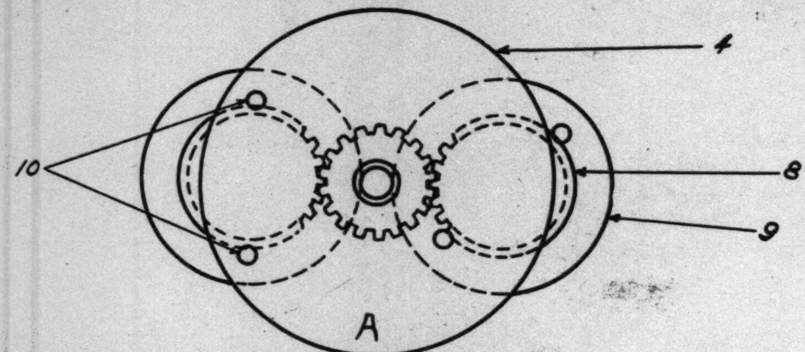
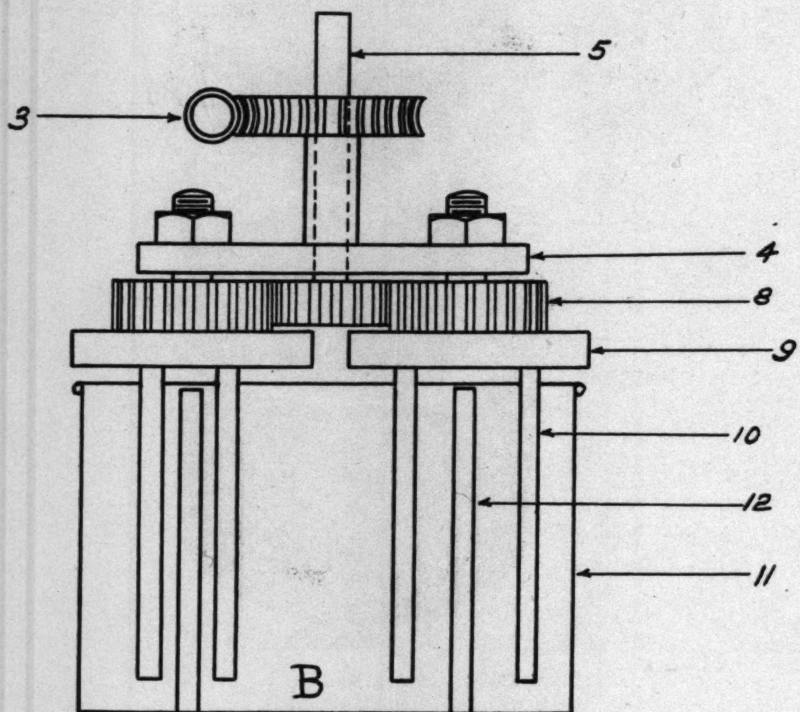


Fig. 1. Mixing Machine

The working parts of the machine are driven by a pulley which connects with a ¼ H. P. motor. By having several pulleys in step it is possible to run the machine at different rates of speed. To this pulley is fastened a balance disc held by friction to steady the motion of the machine. The motor is connected with a wattmeter which makes it possible to measure the input of current while the machine is working.



(A) Gears, Plates, and Arrangement of Pins of Mixing Machine in Horizontal Section



(B) Same in Vertical Section

Fig. 2.

The pulley (1) drives the moving parts of the machine by means of a worm gear drive (3). This worm gear drive turns a hollow shaft fastened to the disc (4). Inside this hollow shaft is the shaft (5) which is held in a fixed position by means of a clamp (6). This clamp is held fixed by the main support (7) to which the principal parts of the machine are fastened. Shaft (5) holds a gear in a fixed position under the disc (4). Disc (4) supports the two gears (8) to which are fastened the two discs (9), and these discs in turn support the four moving pins, (10).

When the disc (4) is turned (clockwise looking down) the two gears (8) turn on the fixed gear under disc (4). This causes the two discs (9) to describe a double or epicycloidal motion, or each disc revolves at the same time as it moves forward. Speed of machine is measured by r. p. m. of disc (4).

The bowl (11) is held in a fixed position by a clamp fastened to its bottom and to the main support (7). To the bottom inside the bowl are fastened 3 pins (12). These pins must be in a certain fixed position with reference to the four movable pins (10).

The two pairs of moving pins (10) alternately straddle and hurdle the fixed pins (12). It is the motion of the moving pins (10) with reference to the fixed pins (12) which constitutes the distinctive characteristic of this machine. When a pair of the moving pins (10) move forward outside the fixed pins (12) the dough is packed and squeezed between the wall of the bowl and the fixed pins. Next, when a pair of the moving pins straddles the fixed pins the dough is pulled and torn.

Figure 2 (A) gives a top view of disc (4), the two gears (8), the two discs (9), and the relation of these to the fixed gear under disc (4). The relation of the moving pins to the discs and gears is shown in section in Figure 2 (B).

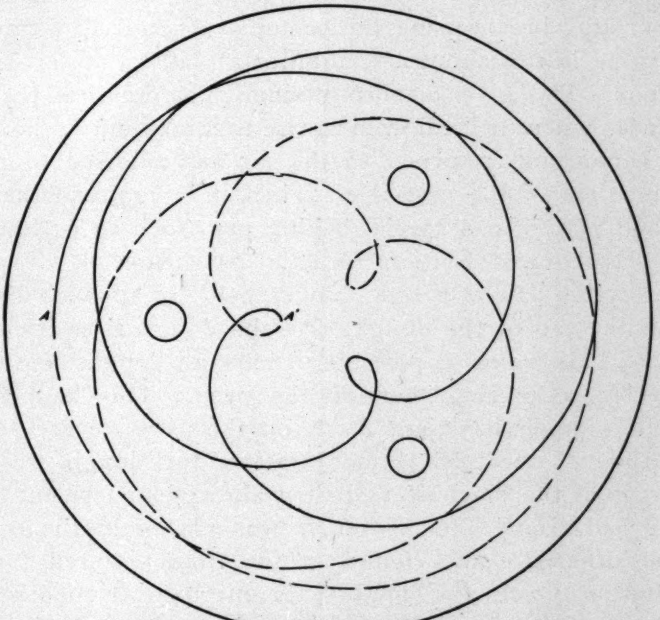


Fig. 3. Path of Moving Pins Inside of Mixing Bowl

Figure 3 shows the path of the moving pins (10) inside the bowl, which is indicated by the outer circle. Each pin moves in the same path as every other pin, but a pin must pass around three times before it begins to retrace its own path. The positions of the three fixed pins are indicated by small circles. In a bowl of this size it would be feasible to have six more fixed pins, three near the periphery and three nearer the center. These additional pins would, however, be of little value and perhaps a detriment. In a larger bowl it would be possible to have three pins nearer the periphery but in line with the fixed pins now provided. What advantage or disadvantage these additional pins would have has not been determined.

Formula Used in Making Baking Tests

The formula used in making baking tests is:

Flour,	grams	340	
Water,	cc.	210	(Exact amount determined by an absorption test.)
Yeast,	grams	10	
Salt,	grams	5	
Sugar,	grams	15	
Lard,	grams	5	

Water at 32°C. is mixed with the yeast, salt, and sugar and the mixture is poured into the bowl of the machine. Then the flour, previously warmed to 32°C., is added, together with the lard, and the machine is started. It must be started slowly or some of the flour will be thrown out. Disc 4 should revolve at about 120 r.p.m. A slower speed was used in some of the first trials.

In making bread, using the customary period of fermentation, the machine is run about 1½ minutes at 120 r.p.m., or 3 minutes at 60 r.p.m. This gives a thoro mixing. The dough is then placed in a tall jar where it is allowed to rise to a maximum, then worked in the hands, and returned to the jar and allowed to rise one-third the time of the first rise. Then it is again worked in the hands and placed in a special baking pan, such as is described in Kansas Experiment Station Bulletin 202, page 14. The special feature of this pan is a rod plunger held by an oval disc which rests on the top of the dough. As the dough rises it pushes up this rod. This makes it possible to have all doughs rise the same amount before placing them in the oven. This happens to be when the plunger has risen 3 cm. on the pans we use. In using the method of mechanical modification, the dough was panned directly from the machine and then allowed to ferment until the plunger had risen 3 cm., when it was placed in the oven. No material difference was found in the time required for panary fermentation using the method of mechanical modification as

compared with the ordinary method using the pre-panary fermentation. The usual time of panary fermentation varies from 45 to 55 minutes.

Experimental Trials with Mechanical Mixing

The data given in the following tables are not presented in the chronological order of performing the experiments. Nor are all the data obtained given, as that would make the paper too voluminous, enough are presented to show the main facts.

In the first experiments the machine was run 60 r.p.m. and the time of treatment varied from 3 to 40 minutes. As soon as the dough had risen the standard amount, or 3 cm., measured on the plunger, the pan was placed in the oven. For comparison the dough for one loaf was mixed 3 minutes and then given the usual two fermentations previous to panning. The results obtained are given in Table I.

TABLE I
EFFECT OF MIXING FOR DIFFERENT LENGTHS OF TIME ON THE QUALITY OF BREAD BAKED WITH ONLY PANARY FERMENTATION

Total time of mechanical treatment minutes 60 r.p.m.	Total time of fermentation minutes	Volume of loaf cc.	Color, score	Texture, score
3	157	1955	95	94
3	61	1690	92	85
6	58	1785	94	88
10	54	1880	96	90
15	54	2045	100	98
20	54	2070	100	99
30	55	2040	100	99
40	55	1990	100	96

Loaf No. 1, mixed 3 minutes and then fermented in the usual way, was in all respects better than loaf No. 2, mixed the same length of time but placed in the pan and baked as soon as the dough had attained the same volume as loaf No. 1. There is no doubt about the advantage of fermentation when the ordinary amount of mixing is used.

The figures for volume, color, and texture show a progressive improvement with the lengthening of the time of mixing. Dough mixed for 15 minutes produced a loaf better in every way than the dough mixed 3 minutes and then fermented. This shows that mechanical action on dough may bring about as favorable results as yeast action.

The effect of mechanical mixing together with the usual two periods of fermentation previous to panning was then tried. The results are shown in Table II.

In this trial the best loaves were obtained when the dough was mixed for from 6 to 15 minutes. In the previous trial the best loaves were obtained when the dough was mixed 15 minutes and longer. It is evident that when the dough is given a severe mechanical treatment, it will not stand the usual amount of fermentation. The decrease in the total time of fermentation of the loaves mixed the longer time was partly due to the fact that the yeast was acting during the mixing process and partly to the weakening of the gluten by the prolonged mixing.

TABLE II

EFFECT OF MIXING THE DOUGH DIFFERENT LENGTHS OF TIME COMBINED WITH THE USUAL TWO PERIODS OF FERMENTATION PREVIOUS TO PANNING

Time of mixing, minutes 60 r.p.m.	Total time of fermentation, minutes	Volume of loaf, cc.	Color, score	Texture, score
1	157	1860	95	94
3	155	1940	95	96
6	149	1965	95	96
10	155	2030	95	96
15	145	1900	95	94
20	140	1860	95	92
30	135	1840	95	85
60	102	1485	95	60

It then seemed desirable to apply this method to different kinds of flour. For this purpose the college flour was used in comparison with that of two other mills and clear flour from the college mill. It was also desirable to know if the addition of ammonium chloride as yeast food is of any advantage, as well as the effect of smaller amounts of yeast. All doughs were mixed 15 minutes (60 r.p.m.) and then panned directly from the machine; except No. 1, which was mixed 3 minutes and then given the usual pre-panary fermentation. The results obtained are shown in Table III.

TABLE III

EFFECT OF MECHANICAL MODIFICATION ON DIFFERENT KINDS OF FLOUR ALSO EFFECT OF ADDING NH_4Cl AND DECREASING THE AMOUNTS OF YEAST

Flour	Loaf No.	Total time of fermentation, minutes	Volume of loaf, cc.	Color, score	Texture, score
College flour	1	154	1930	95	96
" "	2	55	1955	99	99
" "	3	63	2085	99	99
Mill No. 1	4	52	1945	100	99
" " "	5	51	1950	98	96
" " 2	6	50	2045	100	99
" " "	7	45	2070	100	99
Clear	8	50	1790	90	90
"	9	48	1695	90	90
College flour	10*	55	2060	99	98
" "	11†	88	1875	98	96

* 0.4 g NH_4Cl added. † 0.4 g NH_4Cl added, but only 5 grams of yeast.

These figures show that the flours from mills No. 1 and No. 2 made as good bread by this method as the flour from the college mill. They also show that the clear flour was much inferior to the patent. The addition of ammonium chloride was of no particular advantage, and the reduction in the amount of yeast materially increased the time of panary fermentation and reduced the quality of the loaf as measured by texture. It should be said that 10 grams of yeast for 340 grams of flour is very nearly the proportion used by many commercial bakers.

Fifteen minutes is rather a long time for mixing dough. The rate of the machine was, therefore, increased from 60 to 120 r.p.m. After several trials it was found that 7 minutes mixing would produce as good bread as 15 minutes or longer at the slower rate. It was also found that the correct amount of water for the dough was an exceedingly important factor. Either too slack or too dry dough would not give good results. The danger of over mixing was greater when the dough was too dry than when it was too wet.

Results obtained on time of mixing at the higher rate are given in Table IV. In this case all doughs received 205 cc. of water, which appeared to be the minimum for good bread.

TABLE IV
INFLUENCE OF TIME OF MIXING AT 120 R.P.M. ON QUALITY OF BREAD

Time mixed, minutes	Total time of fermentation, minutes	Volume of loaf, cc.	Color, score	Texture, score
3	47	1810	95	88
5	45	1910	97	98
7	48	1985	99	99

Several additional trials indicated that the minimum time necessary is 5 minutes and the maximum 7 minutes, providing the correct amount of water is used. As good a loaf was obtained in several trials with 5 minutes mixing of a dough a little dry, as with more than 7 minutes mixing of a dough that was too slack.

Results obtained by varying the amount of water are given in Table V.

The poorest loaf was obtained with 195 cc. of water and 10 minutes mixing. This result was confirmed several times in different trials. Excessive mixing of too dry dough produces a loaf which has a texture similar to that produced when wheat is bin burnt or stack burnt. The 205 cc. of water produced the best loaf, but an additional 10 cc. did not appear to be too much. The loaf

obtained with 210 cc. showed a slight blackening on the bottom, indicating too strong oven heat, and this probably accounts for the smaller volume.

TABLE V

INFLUENCE OF DIFFERENT AMOUNTS OF WATER ON QUALITY OF BREAD

Time of mixing, minutes	Water, cc.	Time of fermentation, minutes	Volume, of loaf cc.	Color, score	Texture, score
10	195	46	1500	95	80
5	195	49	1740	93	85
7	200	41	1770	95	90
7	205	48	1985	99	99
7	210	43	1880	98	98
7	215	41	1935	99	97

The use of lactic acid has been advocated in connection with short time methods because in such cases the acidity assumed to be necessary does not have time to develop. Standard formulas for short time dough specify 0.4 cc. U.S.P. lactic acid for each pound of bread, and this amount was used as a standard for comparison. The results of using lactic acid are given in Table VI.

TABLE VI

INFLUENCE OF LACTIC ACID ON THE QUALITY OF BREAD WHEN USED IN CONNECTION WITH DIFFERENT AMOUNTS OF WATER

Water used, cc.	Lactic acid used, cc.	Time of fermentation, minutes	Volume of loaf cc.	Color, score	Texture, score
215	0.0	41	1935	99	97
205	0.4	44	1965	99	99
210	0.4	42	2025	99	99
215	0.4	44	1985	98	99
205	0.2	42	1865	97	92
210	0.2	42	1845	96	94
215	0.2	44	1955	97	96
215	0.2	48	1930	95	98
220	0.2	45	2000	98	99

The results indicate an advantage in the use of lactic acid as shown by the slight improvement of loaf volume and texture. The greatest advantage seems to be that a small variation in the amount of water makes less difference when lactic acid is used.

The data presented in Table VII were obtained from two commercial flours and from flour freshly milled, in comparison with the standard flour used in the preceding experiments. All were mixed 7 minutes except loaf No. 3, which was mixed 2 minutes and then fermented and worked down twice before panning. The two commercial flours were not from the same mills as the flours used for the results in Table III.

TABLE VII

Effect of Mechanical Mixing on Two Commercial Flours, and on Flour Freshly Milled

Description of flour	Water, cc.	Total time of fermentation, minutes	Volume of loaf cc.	Color, score	Texture, score
Mill A, 1	200	146	1760	96	90
" 2	200	45	1590	90	80
" 3	210	59	1660	90	82
" B, 1	215	41	2050	100	100
" " 2*	215	44	2060	100	100
College mill 1 mo. old 1	210	44	1820	94	92
" " " " 2	215	45	1880	94	96
" " " " 3*	215	48	1930	95	98
" " " " 4*	220	45	2000	98	99
" a few days old 1	190	50	1605	88	85
" " " " " 2*	200	48	1675	88	88

* Lactic acid was used in mixing the dough for these loaves

The mature college flour was from the standard flour used in several previous experiments. It was about one month old and bleached with Novadel. Flour from Mill A was probably a cut or stuffed straight. Loaf No. 1, Mill A, was from the same flour as Nos. 2 and 3, but the dough was fermented in the usual way. The results show that the usual process of fermentation will produce a better loaf from poor flour than this short method by mechanical action. The amount of water used for this commercial flour was the optimum. This dough went "slack" in the machine, and this indicates weakness. Flour from Mill B was a straight commercial grade, made in a medium sized mill situated in the center of the wheat belt of western Kansas which has the reputation for making strong flour. The freshly ground college flour was of the same grade as the matured. The use of lactic acid did not seem to have as much advantage with the freshly ground flour as with that which was matured.

These results suggest that this short time method with mechanical modification of dough will bring out stronger differences in flour quality than the longer method with the usual time of fermentation.

Experiments in Washing Gluten from Mechanically Modified Dough

When ordinary dough made from mixing wheat flour and water is worked and washed in water, a yellowish rubbery substance known as gluten is obtained. Several trials of washing gluten from mechanically modified dough were made. An attempt was first made to wash the gluten over a piece of bolting cloth.

This seemed to have no advantage except in catching any material accidentally dropped from the hands. After several trials it was found that best results could be obtained by placing a small amount of dough in the hollow of the left hand and working it with the fingers of the right hand. No attempt was made to secure quantitative results. A small amount of dough was taken at different intervals of working the mixer as well as under various other conditions. The following is a summary of observations made.

Gluten from dough just well mixed, which occurs in about 3 minutes with 60 r.p.m. or 1½ minutes with 120 r.p.m. was normal in every way. Gluten from dough mixed 5 minutes with 120 r.p.m. or 10 minutes at 60 r.p.m. manifested a weakened structure. It had lost some of its rubbery strength or resilience. At the end of 5 minutes mixing at 120 r.p.m. the gluten began to go to pieces very rapidly. At the end of 6 minutes of mixing it was very difficult to wash and what was generally obtained had a granular appearance with little or no coherence and no resiliency. At the end of seven minutes, it was as a rule impossible to wash any gluten from the dough. The starch and the proteins seemed to be so intimately mixed that the whole would simply be reduced to a thin gravy when water was added. It appeared as if the gluten strands had been reduced to exceedingly fine meshes inclosing the starch grains. When this has happened, the gluten filaments are probably exceedingly attenuated and hence have little resistance. If a very small stream of water is allowed to fall upon such dough, the fibrous looking filaments can be seen, but they appear short. The starch grains seem to be inclosed in a network of very fine gluten filaments from which it is practically impossible to remove them by washing in water. It also appears that when the gluten strands have been reduced to this condition, they have undergone some structural modification.

It appears that a certain amount of recovery takes place in mechanically treated dough. At the end of 7 minutes mixing at 120 r.p.m. it was seldom possible to obtain any gluten. With a speed of 60 r.p.m. gluten was obtained at the end of 15 minutes, and that obtained at the end of 20 minutes was similar to that obtained at the end of 6 minutes mixing at 120 r.p.m. For mixing at any speed it was easier to obtain gluten if the dough had been allowed to stand a while and then washed, as compared with washing as soon as the dough was removed. This recovery could

be observed in the gluten itself. Granular gluten obtained with 6 minutes mixing at 120 r.p.m. when spread on a watch glass and allowed to stand would regain a small amount of resiliency. At first the band of gluten would spread and manifest no "pull back." After standing some time this "pull back" took place.

Dough mechanically modified is rather sticky when first removed from the machine and can be pulled into long strings. It appears as a mass of stringy material somewhat similar to taffy when the bowl of the machine is lowered. If such dough is smeared on a flat surface, using fingers or spatula, it can be spread into a thin film. Some of the stickiness disappears on standing.

Theoretical Considerations

As the results presented in this paper were obtained by methods very different from usual procedures, it seems desirable to present some of the theoretical considerations which have occurred to us while we have been investigating problems which determine quality in flour. It is recognized that much more work should be done to amplify and substantiate the results of these experiments, but enough has been done to show that there are promising possibilities. An enormous amount of work has been done on the relation of wheat proteins to flour quality. It appears that future work along lines made possible by colloid chemistry is the most promising.

Discussion of Gluten Formation

According to Swanson (1925), dough made from wheat flour is one of the most complex colloid systems. The formation of dough from wheat flour is made possible because of the distinctive characteristics of wheat proteins. These proteins have a strong attraction for water and combine with it in a peculiar way. It is because of the distinctive relationship of these wheat proteins to water that the mixture of wheat flour and water produces a colloid system fundamentally different from that produced by mixing water with the fine sifted meal from any other cereal.

The term "total protein" has acquired a distinct and definite meaning in grain and grain products. The chemist determines the percentage of total nitrogen, multiplies this by a factor, and then calls the result total protein. The term gluten is not always

used with such a definite meaning. In older papers it is especially difficult to know whether the author means total protein, pure protein, or a special form of protein. The word gluten is defined by the Standard dictionary as "A gray tough mass, a mixture of various albumenoids that remains after wheat flour has been washed in water. It gives dough its toughness." If "protein" is substituted for albumenoids and "dough from wheat flour," the definition describes the term gluten in the sense as used by the great majority of cereal chemists. The term gluten then simply means the yellowish rubbery substance which is obtained when a piece of dough is worked and washed in water.

If this meaning of the term gluten is accepted, it is evident that gluten, as such, is formed when water is added to flour. Whether the two proteins, gliadin and glutenin, which compose the bulk of gluten, exist as separate entities in flour or if they are combined when water is added is immaterial to the present discussion. All phenomena associated with gluten formation make it appear that as soon as the water is added the protein colloid particles are drawn together, forming larger aggregates. In the presence of an excess of water, no gluten formation is apparent, but gluten can be obtained from the mixture if the excess of water is removed by centrifugal force. If water in small amounts is dropped on flour, allowing free adsorption, the dough formed is "too wet" to be used in gluten washing. This appears to mean that when flour adsorbs water freely the films of water on the flour particles are too thick to allow the cohesiveness needed in dough from which gluten is to be washed. If such excess of adsorbed water is mechanically removed, gluten can easily be washed from the dough. It appears, therefore, that in order for gluten to form properly, the films of water which cover the colloid protein particles must not exceed a certain thickness.

Gluten formation when water is added to wheat flour may be pictured as follows: The protein colloid particles adsorb water somewhat more strongly than the starch particles and draw together forming larger aggregates. The protein colloid particles behave as tho their structure was in the nature of a "mesh-network." This will account, at least in a measure, for the stronger adsorption of water. The starch colloid particles behave as tho they possessed a more homogeneous internal structure. This would also in a measure account for the less strong adsorption of water. The difference in structure of the protein colloid parti-

cles as compared with the starch particles would account for the fact that the protein particles draw together forming a continuous mesh network in which the starch grains are inclosed. If such dough is kneaded in water, most of the starch is easily washed away, leaving the yellowish rubbery mass which we know as gluten.

Any one who has washed gluten from dough knows that the best results are obtained if the dough is first worked and then allowed to stand a while before the washing begins. This mechanical working assists in bringing the protein colloid particles together into larger complexes. As the forces inherent in the water films surrounding these complexes are strong, the standing allows firmer contacts to form. There are in dough at least two continuous phases, the gluten mesh network which incloses the starch grains and the continuous film of water.

That electrolytes have an influence in the formation of these protein complexes is no doubt true, but it would lead us too far afield to discuss this phase of the phenomena.

How Does Fermentation Modify Dough Texture?

That dough after it has been fermented has physical and chemical properties different from those immediately after mixing in the ordinary way, is well known. It seems to be commonly believed that a part of this change is due to the acids produced during dough fermentation. Johnson and Bailey (1924), however, found that "Acids of fermentation are incapable of accomplishing by themselves the profound changes in the physical properties of the protein which are bound to occur during fermentation." Also "physical properties—elasticity, tenacity, and viscosity—are impaired during the process of fermentation," and "this degradation was due partly at least to proteolytic cleavage of the proteins."

It is also probable that in addition to these chemical agents, the mechanical action of the expanding gas bubbles has an influence in the modification of dough. In the kneading process the aggregates formed by drawing together the protein colloid complexes as soon as water is added to flour in proper proportions, are made to adhere to each other, forming strands which permeate the dough mass. Bread baked from dough kneaded in the ordinary way and allowed only the panary fermentation before baking is

not well "piled".[3] That is, the cell walls are rather thick and the cells large.

When dough is fermented in the ordinary way, these gluten strands are pulled and stretched and hence become attenuated. The first rise is usually to the limit of extensibility. When such dough is worked down, these strands permeate the dough as at first, but in a more attenuated condition. Hence the next rise takes place much faster. It is well known that the manner of working the dough, especially the last time before panning, has a marked influence on bread texture. If the dough is squeezed too much the bread will have a coarse texture similar to that obtained when bread is baked with only the usual panary fermentation. In squeezing, these attenuated strands are again united into larger aggregates. In other words, the mechanical results of dough fermentation have been partially undone.

To get good bread it is necessary that these attenuated strands form a fibrous network which when covered with water forms a membrane capable of retaining the gas when formed. This membrane must not be too strong or too weak. Fermentation is accompanied by proteolysis, which lessens the resistance, and if the strands are properly distributed, and the dough is baked at the right point, the bread crumb will be well "piled." If the fermentation goes too far, the bread will be coarse, which simply means that the gluten network is too weak to hold the expanding gas bubbles, and several coalesce, forming larger cells. Resistance or endurance in prolonged fermentation is a measure of gluten strength. A strong flour will easily come back after an extra "knocking down."

How Does Mechanical Action Modify Gluten?

If the above theory is correct, the following may be offered as an explanation for the mechanical modification of dough by the machine. The alternate pack-squeeze-pull-tear action produces these attenuated gluten strands which permeate the entire dough. The pulling and stretching must be in excess of the "pack-and-squeeze" action. The latter should be just sufficient to make a mass of dough to furnish a hold for the pins which accomplish the pull and stretch. These attenuated strands of gluten form a fibrous network which when covered with water gives membranes

[3] Pile is a term used to describe the relative silkiness and resiliency of the crumb. A well "piled" crumb has small cells, very thin cell walls, and a soft feel, and is resilient.

capable of retaining the gas. The entire action of the machine is such as to give a thoro intermingling of the yeast cells with the entire mass, and each yeast cell as well as starch granule, so to speak, is surrounded by a membrane. The attenuated strands are just weak enough to allow easy expansion, and yet strong enough to retain enough gas to form the porous dough.

When the mechanical action is allowed to proceed too long, it may mean that the strands have become too attenuated and hence are not strong enough to retain the gas, or it may mean that the gluten complexes are so far broken down that they are incapable of forming a membrane sufficiently strong. The difficulty in washing gluten from mechanically modified dough is probably due to the fact that these attenuated strands are very fine, and hence so weak that they can not resist the mechanical force necessary to separate them from the starch cells. Each starch cell, so to speak, is wound with these attenuated strands. That mechanical action in the dry may have a profound influence on flour has been shown by Alsberg and Griffing (1925), who found that "from the most severely ground flour no gluten at all could be washed. In severe grinding the protein colloid complexes are so broken down that they will not unite to form gluten strands of such strength that they are able to resist the mechanical action involved in the washing process.

As there is very little or no proteolytic action in mechanically modified dough, the strands must be made finer than in dough modified by yeast. That such is the case is probable for two reasons: (1) The color of bread from modified dough was better than that of bread from ordinary fermented dough. This simply means finer texture. (2) Gluten can be washed from ordinary fermented dough, but not from mechanically modified dough.

Commercial Use of Mechanical Modification of Dough

Will it be possible to use this method of mechanical modification of dough in the commercial bakery? That the process would save material is obvious, as the time required for fermentation would be reduced by more than one-half. Also, as mechanically modified dough can at once be placed in the baking pan, no dough room would be necessary. This would be a great saving in time and expense. The method would also serve for those who want a "short time dough," as the dough can be placed in the oven in less than an hour after mixing is begun.

In large, high-speed, commercial mixers there is a very rapid increase in dough temperature. This is so serious that the dough must be cooled either by the addition of ice or by cold air. Under the conditions of the experiments presented in this paper no notable increase in dough temperature was observed. It is true that the mass of dough was small and that the structure of the apparatus provides for abundant radiation. At the same time the effective parts of the machine secure the maximum amount of mechanical action on dough with the minimum amount of mechanical motion. This is the important feature in eliminating to a large extent increase in dough temperature.

Another question in the commercial adaptation is the amount of power required. Will the additional power pay? The answer to this question will depend on the value of the saving in time and material due to the shortening of the period of fermentation, and also on the possible economy in the elimination of the dough room.

Irrespective of the commercial adaptation of mechanical modification of dough, these experiments suggest a new angle to study of factors which affect quality in flour. While the use of lactic acid was apparently an advantage, it was not necessary. What relation has this to the pH assumed to be necessary for dough development? It was found in several experiments that aging, bleaching, and drying of flour had an important bearing on the results of mechanical modification. It was also found that the history of the wheat before milling was very important. While several flours were included in these tests, it is obvious that much more work should be done on flours from different kinds and classes of wheat, flours from different mill streams, flours from wheat differently tempered, flours bleached by standard commercial methods as well as degrees of bleaching, and flours stored for different lengths of time and under various conditions.

Summary

1. The experiments presented in this paper show that it is possible so to modify the dough by mechanical means that light bread can be baked using only the fermentation in the pan.

2. This modification of the dough is accomplished by a machine whose action on the dough may be described as pack-squeeze-pull-tear.

3. While the use of lactic acid was an advantage, it was not necessary to accomplish these results.

4. A theory is presented explaining the rôle of mechanical action in modification of dough by yeast action, also how mechanical modification is accomplished by the machine.

5. The value of this method in commercial practice would be measured by the amount of saving of dough material due to shortening the time of fermentation, and elimination of the dough room. This saving would have to offset the added mechanical power required to bring about this modification.

6. Irrespective of the commercial aspects, these experiments show a new point of attack in the study of factors which affect flour quality.

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ERRATA

I Volume III, No. 1, the following corrections should be made:

Page 35, line 29, the word "palmitate" should follow "phytosterol".

Page 53, last two columns of the table, a lower case zeta, ζ, should have been used in the column head instead of the upper case Z to conform to the symbol used in equations 4, 8, 9, 11, and 15.

Page 54, second conclusion should read 19 per cent instead of 9 per cent.

A RAPID METHOD FOR DETERMINING THE GASOLINE COLOR VALUE OF FLOUR AND WHEAT

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(Received for publication February 8, 1926)

Several tests are in use to denote color in flour. Prominent among these are the Pekar test, the tintometer test, tests using the ash content as a basis of color, and the gasoline color test. This multiplicity of tests is due no doubt to the difficulty of expressing the color of flour easily. Flour, being irregular in surface, diffuses light. Moreover, its color is seen by reflected light, thereby making variations among samples and grades harder to detect than if the color could be examined by transmitted light as is the case with fats and oils.

The most popular is the Pekar test. This consists in evenly placing flour side by side, by means of a flour stick or spatula, on some flat surface, such as wood or glass of equal thickness. The board and contents are dipped carefully into water, allowing air bubbles to escape before removing. The color of the flours may be judged while wet, but better after drying a few hours. Specks are plainly brought into relief. The degree of color is estimated by giving a type or standard sample some arbitrary value and comparing other samples against it. Good flour will appear creamy white when wet, and will dry off to a yellowish gray. Poor flour appears a dirty gray and dries off to a dark or whitish gray. It is customary to give patents a value of 100 per cent. A straight may then have a value of 95 per cent, a first clear 80 per cent, a second clear 60 per cent, etc.

In order to maintain a fixed standard of color it is necessary to have a "standard" sample to guide one in making comparisons. This is one of the most serious objections to this method, as the standard bleaches while being kept. Thus a sample of known value becomes paler day by day until it is unfit for further use as a standard. This difficulty is partly overcome by taking flour from the new crop at intervals throughout the year, in order to keep up with the quality. A further objection is the personal equation entering in when reading the color value, no two persons having just the same eye for shades of color. Tests on the same

sample by different individuals have been known to vary by as much as twelve points.

As an alternative to the Pekar test, a tintometer is sometimes employed. A color standard is made of some white material, such as plaster of paris or magnesium carbonate and the color of the flour is matched by the insertion of standard colored glasses of definite units of strength. Objections to this method are that it is slow, is tiring to the eye, and the colored glasses are not always correct and must be checked from time to time. Furthermore, as the units denoting color are very small, the error in judgment is quite large.

A third method is to use the ash content as the basis of color valuation. For every increase or decrease of unit in the ash, a proportionate amount is subtracted from or added to the color value. For example, either a bleached flour with a low ash content is taken for the top of the series, or a standard patent of 0.48 per cent ash may be used for the 100 per cent value. Allowing an increase of one for every decrease of two in the hundredth place of ash, the color of a flour of 0.38 per cent ash would read 105 per cent, one with 0.54 per cent ash would have a color value of 97 per cent, one with 0.72 per cent ash would be rated at 88 per cent. This method, so modified as to keep in line with the color of the flours as they appear on the market, would be as follows: At the beginning of the season when the new crop first appears, a representative average color is taken on the standard patent grade of any particular class of flour and given a value of 100 per cent. The ash is then determined on this sample, or series of samples, the average ash content is taken and thereafter the same addition or deduction is made as above described.

The fourth method, which has come into limited use, is the gasoline color test. A considerable portion of the color imparted to flour originates in the bran and germ. After extracting these materials with a suitable solvent, the color can be measured quantitatively by comparing the gasoline extract with a potassium chromate solution of definite strength. In short, the color of the flour is measured by a fixed and permanent standard. This method appears to be ideal for expressing color, the great drawback being the time consumed in making the test. As now described in the official methods of the Association of Official Agricultural Chemists, an extraction of a 20-gram sample for 16 or more hours with 100 cubic mentimeters of clear colorless gasoline is necessary.

Previous experience in lowering the time limit for determining the acidity of corn[1] suggested vigorous and constant stirring for a short interval of time. A stirrer, Figure 1, such as is used in soda fountains was used. Preliminary tests made it apparent that

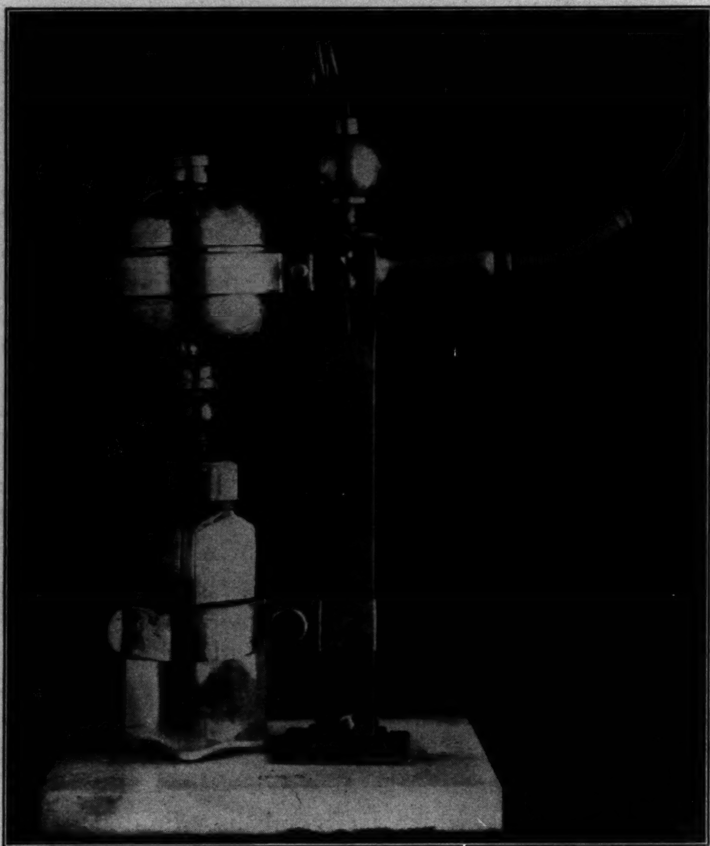

Fig. 1. Mechanical Stirrer with Bottle and Cap in Position

before much progress could be made some means for preventing evaporation was necessary. After several trials, evaporation was cut to a minimum by the development of a metal cap capable of being screwed onto an 8-ounce bottle. A line drawing of this cap is shown in Figure 2.

Preliminary experiments made with this cap showed that by using a 5-gram sample of flour and stirring for 15 minutes, as

1 Improved apparatus for making acidity determinations. Sec. Cir. 68. H. J. Besley and George H. Baston.

great an extraction could be obtained as by using a 10- or 20-gram sample and stirring for 30 minutes. A study was made, therefore, comparing the extraction of 5 grams of flour and 100 cc. of gasoline, and 20 grams of flour and 100 cc. of gasoline stirred with the mechanical stirrer for 15 and 30 minutes, respectively (see Fig. 2) and the results obtained were compared with those obtained by using the method of the Association of Official Agricultural Chemists.[2] Forty-eight samples of flour having a range in gasoline color value from 0.80 to 2.37 were chosen for this comparison. The comparative results are given in Table I.

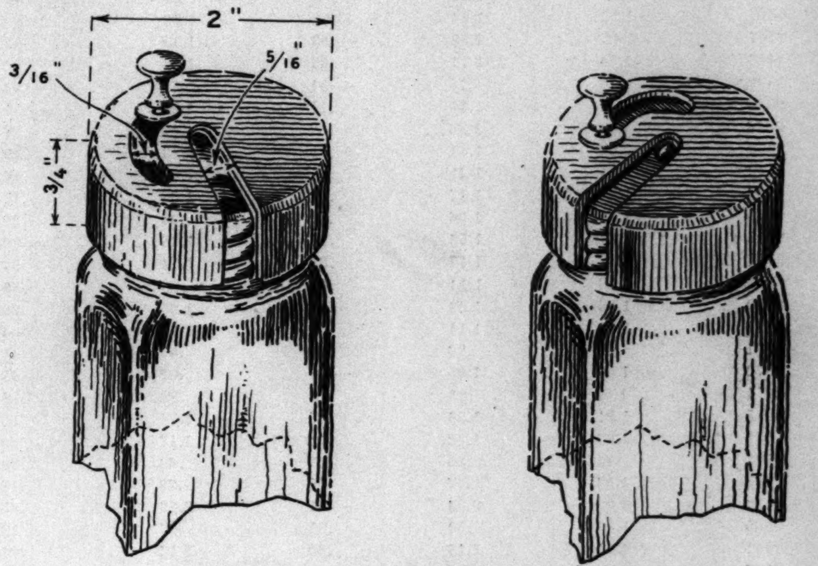

Fig. 2. Metal Cap to Prevent Evaporation

The average difference between Methods 1 and 2 was 0.07, and between Methods 1 and 3, 0.11. The difference between Methods 2 and 3 was 0.04.

It appears from the results of the above tests that for rapid work, stirring 5 grams of flour with 100 cc. of gasoline for 15 minutes gives values similar to those obtained by the method of the Association of Official Agricultural Chemists, in which the time element is 24 hours or more. Closer agreement with the method of the association can be obtained by using 20 grams of flour and agitating for 30 minutes.

2 Official and tentative methods of analysis of the Association of Official Agricultural Chemists, Revised to Nov. 1, 1919. p. 72.

TABLE I

COMPARISON OF THREE METHODS FOR DETERMINING THE GASOLINE COLOR VALUE OF FLOUR

No.	5 grams flour 100 cc. gasoline 15 min. stirring	20 grams flour 100 cc. gasoline 30 min. stirring	Difference between 1 and 2	20 grams flour 100 cc. gasoline Standing 24 hours or more	Difference between 1 and 3
9382	1.99	1.92	.07	2.02	—.03
9383	1.99	1.89	.10	2.02	—.03
9384	2.13	2.16	.03	2.10	—.03
9385	2.25	2.17	.08	2.14	.11
9390	1.90	2.04	—.14	1.95	—.05
9391	.76	.81	—.05	.80	—.04
9392	1.23	1.29	—.06	1.33	—.10
9394	1.16	1.14	.02	1.07	.09
9395	1.53	1.50	.03	1.47	.06
9396	1.26	1.21	.05	1.26	.00
9397	1.32	1.24	.08	1.22	.10
9407	1.42	1.39	.03	1.35	.07
9408	1.74	1.71	.03	1.80	—.06
9409	.76	.77	—.01	.80	—.04
9537	1.47	1.36	.11	1.32	—.15
9538	1.35	1.26	.09	1.09	.26
9539	1.62	1.51	.11	1.43	.19
9542	1.36	1.31	.05	1.20	.16
9564	1.23	1.17	.06	1.06	.17
9565	1.15	1.06	.09	.95	.20
9566	1.57	1.53	.04	1.38	.19
9567	1.18	1.17	.01	1.14	.04
9568	1.18	1.14	.04	.98	.20
9569	1.05	1.03	.02	1.05	.00
9570	1.20	1.11	.09	1.15	.04
9571	.82	.72	.10	.67	.15
9572	1.04	1.05	—.01	.97	.07
9574	1.00	.88	.12	.82	.18
9575	1.91	1.86	.05	1.75	.16
9576	1.27	1.22	.05	1.17	.10
9577	.94	.85	.09	.69	.25
9578	1.06	.97	.09	.88	.18
9579	2.32	2.33	—.01	2.23	.09
9580	1.10	.89	.21	.97	13
9581	2.13	2.13	.00	2.12	01
9582	2.42	2.34	.08	2.37	.05
9583	1.33	1.18	.15	1.17	.16
9584	1.64	1.46	.18	1.40	.24
9585	1.16	1.03	.13	1.00	.16
9717	1.42	1.39	.03	1.42	.00
9718	1.23	1.16	.07	1.15	.08
9719	1.37	1.25	.12	1.25	.12
9720	1.29	1.19	.10	1.16	.13
9721	1.19	1.15	.04	1.00	.19
9722	1.19	.92	.27	.90	.29
9723	1.50	1.41	.09	1.40	.10
9724	1.28	1.17	.11	1.04	.24
9725	1.51	1.45	.06	1.26	.25
Av.	1.42	1.35	.07	1.31	.11
Maximum	....	....	.27	....	.29
Minimum	....	....	.00	....	.00

The method as finally prepared is as follows:

Place 5 or 20 grams of flour into an 8-ounce screw cap bottle, together with 100 cc. of clear colorless gasoline. (If the gasoline

is somewhat yellow, run a blank determination, and deduct from the final reading). Stir with a mechanical stirrer for 15 or 30 minutes, depending upon the size of the sample used.

Filter the extract immediately through a dry double thickness No. 1 Whitman filter paper, or filter paper of similar qualities.

Fill one tube of a Dubosq colorimeter with standard 0.005 per cent potassium chromate solution in water. Fill the other tube with the gasoline extract from the sample under test, and if a 5-gram sample is being used, adjust this tube to the four centimeter mark on the tube scale. If a 20-gram sample has been used, adjust the tube scale to one centimeter.

By adjusting the standard tube until the colors in each tube match the gasoline, color value can be read correctly from the scale in the standard tube.

The gasoline color is then read direct, using one centimeter as unity. Any other form of colorimeter may be used to obtain comparable results with the new method if it is kept in mind that the extract is one-fourth as strong as that obtained by the method of the Association of Official Agricultural Chemists. With a battery of six shakers, six samples may be run in 30 minutes.

This method has also been used to advantage for determining the gasoline color value of wheat samples.

In order to secure good results with ground wheat samples, it is necessary to grind the whole wheat so that 75 per cent of it will pass through No. 50 grits gauze. Otherwise, the procedure is the same.

The use of wheat instead of flour has been found to be a decided advantage in a study of the color of wheats from a plant breeding standpoint.

WHEAT AND FLOUR STUDIES VI
EFFECT OF YEAST FERMENTATION ON THE PROTEINS OF FLOUR[1]

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INTRODUCTION

A brief review of some of the investigations that might have a bearing on the changes which take place during yeast fermentation was given by Sharp and Gortner (1924). These authors then made a brief study of the changes in the imbibitional properties of the gluten during yeast fermentation. They took samples from the dough, dispersed them in distilled water, extracted the electrolytes, and measured the maximum so-called viscosity on the addition of lactic acid. Sharp and Gortner (1923) used this method for measuring the imbibitional properties of the flour proteins and found that glutenin was the protein mainly responsible for the increase in imbibition on the addition of lactic acid. Yeast was found to produce a marked change in the imbibitional properties of the glutenin as fermentation progressed, the imbibition rising to a maximum and then decreasing. In experiments in which malt flour and malt extract were added to the yeast dough, the maximum was not so high, and when different amounts of malt flour were added the increase in imbibition produced by the yeast was less the greater the amount of malt flour added. Doughs containing all the ingredients except yeast showed little change in their imbibitional properties on standing. It was also found that as yeast fermentation progresses it becomes impossible to obtain thoroly washed gluten from the dough using distilled water, altho gluten could be washed from the dough if sufficient electrolytes were present in the wash water.

Bailey and Johnson (1924), in continuing along the line of investigation begun by Bailey and Weigley (1922), describe two methods for determining the loss of carbon dioxide from the fer-

1 Published with the approval of the Director.

2 This work was presented as an undergraduate thesis in June, 1923, to the Chemistry Department of the Montana State College, by Miss Olive M. Schreiner.

menting dough. They found that after a lapse of from 100 to 180 minutes, the loss in carbon dioxide from the dough rapidly increased and they use this point as an indication of the optimum fermentation period.

Olsen and Bailey (1925) give a review of the literature dealing with the question as to whether or not yeast secretes proteolytic enzymes. These authors then investigated the question for themselves and came to the conclusion, "that the proteases contributed by sound, normal intact yeast cells (baker's yeast) are negligible in their effect upon the properties of gluten during a 4- or 5-hour fermentation period." In their investigation, "35 grams of flour was placed in a flask and 125 cc. of water previously warmed to 30° C. was added. After the suspending of the flour particles by agitation of the mixture, 15 cc. of a 10 per cent suspension of yeast in distilled water was added and thoroly mixed with the flour and water. The flasks were incubated, with occasional shaking, for the desired time at 30° C. Controls were prepared in the same manner except that 140 cc. of water was used and no yeast added." The viscosity of 75 cc. of the suspension on the addition of 6 cc. of N/1 lactic acid was determined in a MacMichael viscometer. They found that as fermentation progressed, the viscosity decreased. The decrease was apparently due to the effect of acid developed by the yeast, for the same decrease could be obtained by adding enough acid to a flour suspension containing no yeast to produce the same change in hydrogen-ion concentration as was produced by the yeast, and determining the viscosity after various periods of time. Also, if alkali was added to the yeast-fermenting suspension at frequent intervals so as continuously to neutralize the acid produced by the yeast, then the decrease in viscosity as fermentation progressed was largely prevented.

The results obtained by Olsen and Bailey (1925) are in apparent disagreement with those of Sharp and Gortner (1924). The procedures followed were, however, entirely different. Olsen and Bailey used a simple flour-in-water suspension with a ratio of about 1 to 4, while Sharp and Gortner used a regular dough with a ratio of about 1 to 0.6, to which also were added salt, sugar, and lard. The fermentation temperature was 27° C. Sharp and Gortner removed the greater part of the extractable electrolytes before adding the lactic acid, while Olsen and Bailey did not. Olsen and Bailey found that the conductivity of the suspension to which yeast was added did not at all increase in proportion to the decrease in viscosity

and that the conductivity of the flour suspension to which no yeast was added also increased at even a somewhat faster rate, while showing no decrease in viscosity. Also Olsen and Bailey found that progressive solution of the protein occurs as the acidity increases and they state that this change is reversible.

Olsen and Bailey found only slight increases in the simple nitrogenous compounds as fermentation progressed, and they conclude that no substantial increase took place.

A great number of investigators have determined the loss in dry matter due to yeast fermentation. The amount of dry matter lost as given by these different investigators varies greatly, probably because of differences in fermentation time, amount of yeast, activity of yeast, availability of carbohydrates, temperature, and other factors. Snyder (1897) found an average loss of 2.10 per cent dry matter for straight doughs of short fermentation, i. e., 2½ hours, and an average loss of 8.08 per cent for sponge doughs of what he calls long fermentation, i. e., average 27 hours. He also reports a loss of nitrogen amounting to 1.74 per cent in doughs of short fermentation and 7.77 per cent in those of long fermentation. Later, Snyder (1899) actually determined the loss of carbon dioxide from dough by collecting it in alkali and weighing. He found on the average a loss of about 1 per cent of the weight of the flour, and considering that 1.04 parts of alcohol should be produced at the same time, the total loss in dry matter would amount to about 2 per cent. He also found that other volatile organic compounds were lost which yielded about 0.10 per cent as carbon dioxide. In his second series of determinations he found a loss of 1.8 per cent of dry matter and 1.45 per cent of the total nitrogen in the short fermentations, and a loss of 5.94 per cent of dry matter and 6.75 per cent of the total nitrogen in the long fermentations. Snyder bubbled the gases given off from the dough through sulfuric acid and later determined the nitrogen in the sulfuric acid by the Kjeldahl method. He found that 0.02 per cent of the total nitrogen of the dough was present in the sulfuric acid. He found that even a greater amount was present in the sulfuric acid when the gases produced in baking were bubbled through it. Also that when bread was dried in the drying oven a greater amount of nitrogen was given off than in the baking process.

Voorhees (1900) found the loss of dry matter to vary from 1.41 to 3.76 per cent, depending on the treatment of the dough; and the fluctuation of protein to vary from —0.22 to +0.08 per

cent of the weight of the flour. These doughs would probably correspond to Snyder's short fermentation doughs.

Bremer (1907) states that yeast fermentation produces no change in the properties of the gluten.

Jago and Jago (1921 a) also investigated the loss in dry matter due to fermentation and found a loss of 2.5 per cent in from 8 to 10 hours at a temperature of 85 to 90° F. They found that the loss was less if more yeast was added.

Jago and Jago (1921 b) studied some of the changes produced in yeast dough during fermentation. They studied changes in both proteins and carbohydrates. Their results are rather difficult to interpret, and bring out what we later found to be true, that is, that the quantitative determination of the protein in bread dough offers considerable difficulty.

Experimental

The effect of the yeast on the imbibitional properties of the glutenin, as shown by Sharp and Gortner (1924), was so pronounced that it was thought advisable to analyze a yeast dough as fermentation progressed in order to see if we could detect any difference in the protein fractions as shown by solubility analysis.

The flour used was a baker's patent milled from Montana spring wheat.

The formula used in preparing the dough was as follows:

	Grams	Per cent
Flour	340.0	100.0
Water	204.0	60.0
Yeast	13.6	4.0
Sugar	10.2	3.0
Salt	5.1	1.5
Lard	6.8	2.0

The various ingredients were weighed accurately and the sugar and salt dissolved in the water. All ingredients were placed in suitable containers, and immersed in a water bath at a temperature of 27° C. until they attained the temperature of the bath. The yeast was then suspended in the sugar-salt solution, the flour and lard were added, and the dough was mixed. The dough was weighed, samples were removed for analysis, and the dough was again weighed. The dough was then returned to a loosely covered fermentation jar immersed in the water bath.

The samples of dough removed for analysis were as follows:

1. A 10-gram sample for moisture determination. After drying, this sample was also used for crude protein determination.

2. A 4-gram sample for the determination of the amino nitrogen content.

3. A 10-gram sample for the determination of the potassium sulfate-soluble proteins followed by the determination of the alcohol-soluble proteins in the residue.

4. An 8-gram sample for the hydrogen ion determination.

At the end of every hour the dough was kneaded and weighed, samples were removed for analysis, the dough was again weighed, and returned to the fermentation jar. Samples were removed every hour for ten hours and one set of samples was removed at the end of 24 hours.

The analytical methods used were as follows:

1. Moisture and crude protein determination. The 10 grams of dough removed for this determination was equivalent to approximately 6 grams of the original flour. The weighed sample was placed in a moisture dish and dried in a vacuum oven at 100° C. and again weighed. The dried material was then ground, duplicate 1-gram samples were taken for the determination of total crude protein (N X 5.7), and the moisture was determined in the remainder to correct for the moisture taken up by the samples in the grinding process.

2. Amino nitrogen. The 4-gram sample of dough taken for this analysis contained only slightly less than 2.5 grams of the original flour. The sample of dough was mascerated in a mortar with a few cc. of water, washed into a 25 cc. volumetric flask, and shaken vigorously with about 12 cc. of water and 1.25 cc. of a solution containing 20 grams of sodium tungstate in a volume of 100 cc. The material was shaken until the dough was dispersed, then 4 drops of concentrated sulfuric acid were added and the flask was made up to volume and again shaken. This material was centrifuged and the amino nitrogen determined in a 2-cc. aliquot of the supernatant liquid, using the micro Van Slyke apparatus.

3. Potassium sulfate-soluble protein, followed by alcohol-soluble protein on the residue. The 10-gram sample of dough contained approximately 6 grams of flour and 3.6 of water. This sample was mascerated in a mortar with a few cc. of water, placed in a centrifuge bottle, 50 cc. of 10 per cent potassium sulfate added,

and then enough water added, including that added in the mortar, to make 46.4 cc. The bottle was shaken vigorously until the dough was dispersed and it was then placed in a mechanical shaker and shaken for one hour. At the end of this time the material was centrifuged and the protein in a 50-cc. aliquot determined by the Kjeldahl method. The remaining supernatant liquid was decanted into a 50-cc. graduated cylinder. The residue was treated with 100 cc. of 70 per cent alcohol plus an amount of alcohol equivalent to the amount of supernatant liquid decanted into the graduated cylinder. Therefore the residue, equivalent to about 6 grams of flour, was treated with 150 cc. of approximately 70 per cent alcohol and shaken with the alcohol until it was dispersed and then shaken continuously in a mechanical shaker for one hour. At the end of this time it was centrifuged and the protein in two 50-cc. aliquots determined by the Kjeldahl method. In all cases the nitrogen was converted to protein by the use of the factor 5.7.

4. The dough taken for the hydrogen ion determination was mascerated in a mortar with 25 cc. of distilled water and the pH of the mixture was determined electrometrically.

The results were finally calculated to the basis of the moisture-free material, containing the original amount of dry matter. The results obtained are given in Table I. Table I, column 2 indicates that the moisture content of the dough remained nearly constant throughout the period. As the dough was weighed every time a group of samples was removed, data were available for calculating the loss of dry matter due to fermentation. The values of column 2 were recalculated to the percentage of the original weight of the dough present as dry matter at the end of the various periods of fermentation. The results of this calculation are given in column 3. Column 4 gives the loss of dry matter due to fermentation. The percentage of protein (N X 5.7) in the dry samples of column 2 is given in column 5. The results in column 5 show a gradual increase as fermentation progresses, but if these results are corrected for the loss of dry matter due to fermentation, the protein content given in column 6 is obtained. This serves as a check on the calculation of the fermentation loss as the protein values are approximately constant or decrease only slightly. The protein extractable with 5 per cent potassium sulfate is given in column 7. The results are rather erratic yet they show no definite increase in the potassium sulfate-soluble fraction during what would be the normal fermentation period and only

possibly a slight increase after longer periods. The 70 per cent alcohol-soluble protein of the residue, column 8, apparently shows a slight decrease in the middle of the column, but it is doubtful if the differences exceed the experimental error. The glutenin, column 9, is obtained by difference, and here the errors found in columns 6, 7, and 8 are operative. The amino nitrogen, column 10, seemed to decrease and at the end of the period to increase again. The pH, column 11, showed a decrease as fermentation progressed.

The results, while rather erratic, seem to justify the conclusion that baker's yeast produced no appreciable changes in the proteins of the flour so far as this method of protein solubility fractionation shows, during the period of normal fermentation. It will also be noted that this experiment was carried out with a 4 per cent yeast dough. Thus an amount of yeast was used which was considerably in excess of that used in normal commercial baking.

The main reason for the erratic results was probably the difficulty of preparing a suspension of the dough which contained no lumps. A duplicate series of determinations was carried out in which the sample of dough was ground with a solution of potassium hydroxide and when the dough was apparently dispersed, an equivalent of sulfuric acid was added. It was necessary then to add enough potassium sulfate to make the solution 5 per cent with respect to the salt. The determinations from this point on were the same as previously described. The results obtained by this method were not so uniform as those given in Table I. The main difficulty seemed to be so to neutralize the alkali as to bring the hydrogen-ion concentration back to the starting point. The results obtained by this alkali method taken as a whole seem to confirm the previous statement that no difference in the protein solubility fractions was produced by the yeast during the period of normal fermentation.

As Sharp and Gortner (1924) had found the marked increase in the imbibitional power of the glutenin as fermentation progressed, it was thought that if doughs were prepared alike in all respects except that different amounts of yeast were added, this effect of the yeast might be correlated with the amount of yeast in the dough. Doughs were prepared containing no yeast, 1 per cent, 2 per cent, and 4 per cent, using the dough formula and procedure already described.

TABLE I

PROTEIN SOLUBILITY FRACTIONS IN DOUGH AFTER VARIOUS PERIODS OF FERMENTATION, 4 PER CENT YEAST USED

	Dry matter			Total crude protein, dry basis		Protein (Nx5.7) solubility fractions dry basis corrected for loss in dry matter due to fermentation.				
Time intervals, hours	in sample taken at time intervals	after correction for loss in dry matter of dough	loss due to fermentation	in samples in column two	corrected for loss in dry matter due to fermentation	K_2SO_4 soluble	alcohol extract of K_2SO_4 residue	residue by difference, principally glutenin	Amino nitrogen	Dough
1	2	3	4	5	6	7	8	9	10	11
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	pH.
Calculated	56.7	56.7	0.0	12.96	12.96	....	....	....	....	....
0	56.8	56.8	0.0	13.28	13.28	1.98	5.96	5.34	.033	5.42
1	56.4	56.1	1.2	13.38	13.23	1.93	5.62	5.68	.000	5.36
2	56.6	56.0	1.4	13.41	13.22	1.92	5.90	5.40	.004	5.26
3	56.5	55.4	2.4	13.44	13.12	1.83	5.25	6.04	.000	5.19
4	56.2	54.8	3.5	13.64	13.16	1.88	5.22	6.06	.008	5.10
5	56.4	54.5	4.0	13.75	13.20	1.89	5.20	6.11	.000	4.99
6	55.9	53.7	5.4	13.80	13.06	1.93	4.94	6.19	.008	4.97
7	55.8	53.2	6.3	13.88	13.01	2.16	5.34	5.51	.000	4.93
8	55.7	52.7	7.2	13.95	12.95	2.13	5.62	5.20	.000	4.95
9	55.6	52.2	8.1	14.14	12.99	2.15	5.75	5.09	.000	4.92
10	55.6	51.8	8.8	14.17	12.97	2.25	5.51	5.21	.018	4.91
24	55.7	50.8	10.5	14.65	13.12	2.00	6.12	5.00	.027	4.34

The apparatus described by Sharp (1925) for the investigation of flour-in-water suspensions as plastic solids was used. Samples of dough were taken at various time intervals and their flow through the plastometer was measured. The procedure followed in preparing the suspensions was to take a 50-gram sample of the dough, grind it in a mortar with water to disperse it, dilute the mixture to 500 cc. and shake. At the end of 5 minutes the

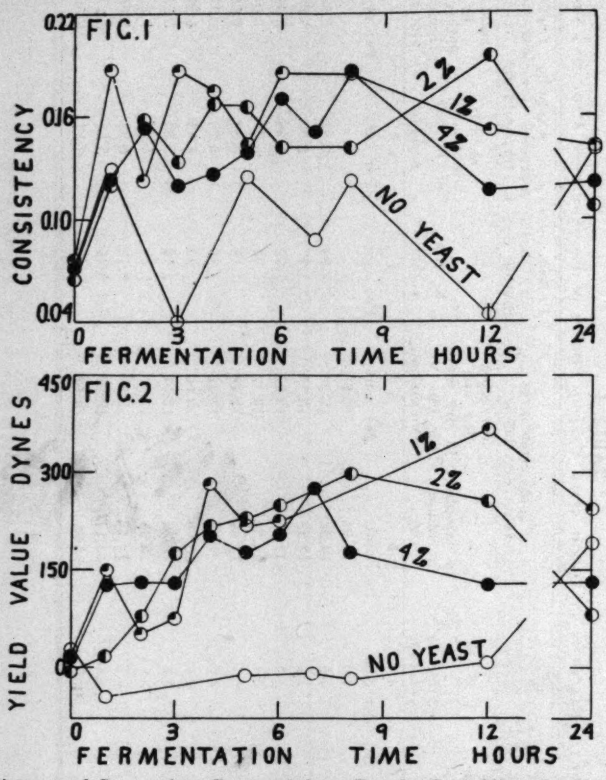

Fig. 1. Consistency of Suspensions Prepared from Doughs Containing the Various Amounts of Yeast Indicated, after Different Periods of Fermentation

Fig. 2. Yield Values of Suspensions Prepared from Doughs Containing the Various Amounts of Yeast Indicated, after Different Periods of Fermentation

supernatant liquid was decanted and the residue treated with an additional 500 cc. of water. This treatment was performed four times, so that the material was extracted with 2 liters. After the final extraction the residue was made up to a volume of 200 cc, 4 cc. of normal lactic acid was added, and the mixture was shaken for 10 minutes in a mechanical shaker. The flow was then determined according to the method described by Sharp (1925). The resulting flow in cc. per second was plotted against the shearing

stress. The yield value and the consistency were then determined by the graphical method. In some instances no definite straight portion of the curve was apparent. In these cases the line was drawn approximately as indicated by the upper points. The values of the constants for the various time intervals are given in Table II. The results are expressed graphically in Figures 1 and 2. In Figure 1 the consistency values as obtained with the different doughs are plotted against time of fermentation. The doughs containing yeast show higher consistency values than that without yeast throughout the entire period except at the end of 24 hours, when the no-yeast dough also shows a consistency comparable with the yeast dough. This higher value of the no-yeast dough at the end of the 24 hours is not inconsistent, for, from the 10- to the 24-hour period the no-yeast dough about trebled in volume, owing to the development of organisms naturally present in the flour or collected from the air. It could not be considered as true no-yeast dough at this period, but as yeast dough, and it showed the effects of yeast on the imbibitional properties of the glutenin at this period.

In Figure 2 the yield values are plotted against time of fermentation. In the yeast doughs the yield values increase with time up to a maximum and then tend to fall off at the 24-hour period. The yield values of the no-yeast dough tend to remain about zero until the 24-hour period is reached, when the yield value of the no-yeast dough increases to a value comparable with the yeast doughs. This increase is not inconsistent, however, for the no-yeast dough showed fermentation at the end of 24 hours, as mentioned, and consequently a higher yield value would be expected.

These experiments confirm the earlier findings of Sharp and Gortner (1924) that yeast apparently in some manner causes an increase in imbibition of the glutenin, from which the salts are largely removed, on the addition of lactic acid. It seems that the yeast increases both the consistency and the yield value, altho the yield value is apparently increased to a greater extent than is the consistency. At the end of 24 hours, the no-yeast dough showed considerable fermentation due to the development of organisms present in the flour or collected from the air, and the effect of these organisms was evidenced in the imbibitional properties of the glutenin. It will be noted in Table II that the 4 per cent yeast dough extract showed a very low so-called consistency at the end of 192 hours.

TABLE II

Change of Plasticity Values with Progress of Fermentation in Doughs Containing Various Amounts of Yeast

Time, hours	No Yeast: Yield value, dynes	No Yeast: Consistency	1% Yeast: Yield value, dynes	1% Yeast: Consistency	2% Yeast: Yield value, dynes	2% Yeast: Consistency	4% Yeast: Yield value, dynes	4% Yeast: Consistency
0	+27	.073	+16	.074	—8	.065	+12	.072
1	—47	.130	148	.187	+16	.121	124	.126
2	....	....	51	.122	78	.158	128	.157
3	+140	.037	74	.187	171	.184	128	.120
4	....	....	276	.176	214	.167	198	.127
5	—12	.125	214	.142	226	.165	175	.139
6	....	....	222	.185	245	.141	202	.169
7	—8	.089	...	....	...	....	272	.152
8	—16	.122	505	.184	295	.140	175	.188
12	+8	.045	362	.152	253	.195	128	.117
24	+187	.140	237	.141	78	.108	128	.121
192	....	....	...	....	...	....	...	.0132

Conclusions

1. Baker's yeast during normal dough fermentation produces no marked changes in the proteins of the flour so far as the method of solubility analysis used here shows.

2. The plasticity constants, as represented by the consistency and the yield value of glutenin suspensions which have been treated with lactic acid after the removal of the electrolytes, increase to a maximum as yeast fermentation progresses. The increase in the yield value is the more apparent.

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EFFECT OF MONO CALCIUM PHOSPHATE UPON THE VISCOSITY OF ACIDULATED FLOUR-IN-WATER SUSPENSIONS

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For many years various flour improvers have been added to flour with the result that the volume of the loaf of bread has been increased and the color improved with no injurious effect on the texture. One of the best known of these so-called improvers is mono calcium phosphate.

Our purpose in this work was to study the effect produced by phosphating various flours, on the viscosity of the acidulated suspension and on the quality of the flour.

Sharp and Gortner (1923) have shown that the addition of various salts to the acidulated suspension decreases the viscosity of the suspension. We have been unable to find any record in the literature of the effect on the quality of flour by the addition of phosphate.

The types of flour selected for this work were chosen to give a wide range of quality with which to work. All the flours were made from wheat of the 1924 crop, were milled in April, 1925, and stored until May, when the studies were made.

The three flours studied will be called A, B, and C. Flour A is a 100 per cent flour milled from high protein hard winter wheat. Flour B is a 100 per cent flour milled from a No. 1 hard winter wheat of 12 per cent protein content. Flour C is a soft wheat patent milled from No. 2 Soft Red Winter wheat. It was not possible to obtain a 100 per cent flour of this type.

As all were plain flours, it became necessary to blend in the correct amount of phosphate for each mixture. The mono calcium phosphate was used in various amounts according to the strength of the flour considered. This was uniformly done, as was shown by the ash test made upon the samples.

The method of Sharp and Gortner was used for measuring the viscosity and determining the quality factor, except that instead of having four or five different concentrations for each flour we used only two and chose these so that a wide range in viscosity readings resulted. In calculating the factor it is obvious that the

method of least squares would not apply because of limited data. However the tangent of the curve is easily calculated from two points. We have checked the results from this method with those from methods using more concentrations and find that the results are reliable and check very well. This is the method used in our routine work. Electrolytes were removed by washing and viscosity of an acidulated suspension made to 100 cubic centimeters was determined. The data obtained, together with the analysis of each flour, are given in Table I.

TABLE I

Composition and Viscosity of Three Flours Containing Various Quantities of Mono Calcium Phosphate

Flour	Phosphate per cent	Ash per cent	Crude protein per cent	Viscosity 16 Grams °Mac M.	Viscosity 10 Grams °Mac M.	Quality factor
A	0.00	0.48	13.00	474	143.0	2.58
A	0.50	0.89	13.00	374	113.0	2.55
A	1.00	1.33	13.00	330	96.0	2.61
A	1.50	1.73	13.00	300	88.0	2.61
A	2.00	2.14	13.00	269	81.0	2.56
B	0.00	0.47	11.00	326	94.0	2.64
B	0.25	0.69	11.00	270	77.0	2.67
B	0.50	0.90	11.00	248	70.0	2.60
C	0.00	0.32	8.50	204	33.3	3.86
C	0.50	0.75	8.50	135	21.4	3.90
C	1.00	1.15	8.50	88	14.4	3.84

The viscosity determinations were made with the MacMichael viscosimeter using a No. 28 wire for flours A and B. With flour C the determination was made with a No. 30 wire and the results were calculated to that of a No. 28 wire and are so recorded in Table I.

The results obtained in this work show, as has been previously shown by Sharp and Gortner, that the addition of mono calcium phosphate to the flour will decrease the viscosity of its flour-water suspension. The viscosity decreases as the concentration of phosphate increases. However, we do not find any definite relation between the decrease in viscosity per unit increase in the concentration of phosphate. It shows clearly that the change is greatest in all flours for the first addition.

We find no appreciable change in the quality factor due to phosphating of flour. We have never been able to obtain the same relative differences between the quality factor of soft and hard wheat flours as that shown by Sharp and Gortner.

In our routine work we have found that the factor for a hard winter patent is about 3.00. This is in line with the results of

Sharp and Gortner. However, as shown above, we find a higher factor for soft wheat flour, in this case 3.84 and 3.90, while their factor for soft red winter was 2.398.

The fact that the quality factors for the various concentrations of phosphate show a slight variation can be partially explained, as the phosphate was blended with the flour by hand and a slight variation might result in each concentration. Also the magnitude of the various viscosity readings might be considered a source of error which would affect the factor.

In conjunction with the work on viscosity and the calculation of the quality factor, we thought it advisable to make a baking test of each of the flours, both plain and in the various concentrations of phosphate. This gave us some very interesting data as to both color and loaf volume. The data obtained are given in Table II. All baking tests were made from 340 grams of flour. The same amount of ingredients was used in baking regardless of the percentage of phosphate added. However, less yeast was used in baking the soft wheat flour.

TABLE II

RESULTS OF BAKING TESTS OF PLAIN AND PHOSPHATED FLOURS

Flour	Phosphate per cent	Absorbtion per cent	Fermentation period minutes	Loaf		
				Color	Texture	Volume
A	0.00	66	145	100	100	2325
A	0.50	66	145	102	102	2650
A	1.00	66	145	104	104	2800
A	1.50	66	145	101	100	2350
A	2.00	66	145	98	98	2050
B	0.00	61	135	100	100	2750
B	0.25	61	135	98	98	2575
B	0.50	61	135	96	96	2500
C	0.00	52	130	100	100	2525
C	0.50	52	130	97	97	2000
C	1.00	52	130	90	90	1600

From the results of our baking test, we find that for bread-making purposes, flour A was the only one of the three that could be phosphated to advantage. This flour, containing a high percentage of protein of good quality, could be phosphated to the amount of 1 per cent.

Flour B gave the best results in the plain flour. Flour C, as was to be expected, would not bake when phosphated. The doughs for this flour in the 1 per cent concentration broke very badly in the proof. However, flours of this type are very frequently phos-

phated and used for biscuit making and show an improvement over the unphosphated flour for this purpose.

The viscosity of flour A, in the concentration at which maximum loaf volume was obtained (Table I), is 330 degrees. For flour B (a plain flour) the viscosity at maximum loaf volume was 326 degrees. With flour C the maximum loaf volume was obtained from the plain flour which had a viscosity of 204 degrees. Our results would then tend to show that flours possessing a higher viscosity above 330 degrees might be phosphated to advantage for bread making altho our data are not adequate to lead to any definite statements on this point.

In connection with this work an ash determination was made upon the dried suspension of each flour. Previously we had made ash determinations upon the dried suspensions and found that after washing to remove the electrolytes the ash content was about one-half that of the original flour.

However, it was found to be impossible to make an ash determination upon the suspension used for viscosity, owing to the very slow filtering of the acidulated suspension. The time required to filter and dry was two days and the danger of contamination was too great to give accurate results.

To prepare the suspensions, of both the plain and the 1 per cent phosphated flours, we washed out the electrolytes with two portions of distilled water, 100 cc. and 500 cc. respectively. At the last decantation as much water as possible was decanted and the suspension was allowed to drain into an ashless filter and was then dried. The ash content of each dried suspension and upon a blank using five filter papers was determined.

It is evident that any increase in the ash content of the dried suspension might be due to the insoluble impurities of the mono calcium phosphate, as commercial phosphate was used in this work. In order definitely to establish our findings it was necessary to remove the insoluble matter. To do this we dissolved the amount of phosphate to make 1 per cent of the flour by weight in one liter of distilled water. After the phosphate was dissolved the solution was filtered and the plain flours were treated with the solution instead of with distilled water. In order to remove all traces of phosphate, the residue after decantation was washed twice with distilled water and the water decanted. The residue was then poured into a filter and the ash determined as before. The ash determined by both methods is given in Table III.

TABLE III

ASH CONTENT OF NATURAL AND PHOSPHATED FLOUR AFTER WASHING THE FLOUR WITH WATER AND WITH PHOSPHATE SOLUTION

	Washed with water		Washed with phosphate solution
	Ash of natural flour	Ash of phosphated flour	Ash of natural flour
A	0.286	0.381	0.280
B	0.260	0.352	0.260
C	0.204	0.243	0.200

These determinations tend to show that there is more ash in the suspension from the phosphated flour than in that from the plain flour. This increase in ash could not have resulted from an excess of phosphate over the amount which would have been dissolved by the water used in washing. Evidently the phosphate ion must have entered into combination with the protein as it was not free to dissolve and be removed by the wash water.

Summary

1. Mono calcium phosphate lowers the viscosity of the acidulated flour-in-water suspension but does not affect the quality factor.

2. Mono calcium phosphate reacts with the protein of the flour as shown by the increase in the ash content of the dried suspension.

3. The dried suspension represents 65 per cent of the original flour used.

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CONTROL OF DIASTATIC ACTIVITY IN WHEAT FLOUR. I. PRODUCTION OF DIASTATIC FLOUR AND EFFECT OF LARGE DOSAGES.[1]

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Introduction

Improvement in the quality of manufactured products, particularly flour, has been the constant aim of the wheat miller. Likewise, the baker has sought to bring about improvement in the foods baked from flour. A comparison of twentieth century milling methods with those of a half century ago leaves no doubt concerning the advances made by the milling industry. Perfection has not been reached, however, and research leading toward the betterment of mill products has by no means been discontinued. In spite of the marked progress in design of mill machinery and development of more refined methods for milling wheat, the miller is still circumscribed in his efforts to produce flour of high quality by the characteristics of the raw material with which he works.

Quality in flour has come to be recognized as a complex characteristic, or rather a combination of several complex factors. Flour strength, the historic definition for which is the ability of a flour to produce large well-piled loaves (Humphries, 1905), can not be ascribed to a single chemical compound or to the physical condition of any chemical entity, but must be considered as a combination of many factors, the proper combinations of these factors determining very largely the quality of the flour.

For many years research has been directed by almost a multitude of investigators toward the determination of those characteristics, whether chemical or physical or both, which are responsible for the strength of wheat flour. In part these efforts have been directed toward the establishment of methods for evaluating flour which will yield results of greater exactitude than the baking test. Many theories have been advanced, some of which have been

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discarded; nevertheless much information has been accumulated, with the result that in the light of our present knowledge we recognize certain desirable attributes and can also recognize certain deficiencies. Attempts have been made to overcome some of these deficiencies with varying degrees of success.

Certain sections of the wheat-producing areas have for some time been known to yield wheats which are usually superior in bread-making value. Within these territories there have been noted some localities producing wheat which is ordinarily sound, plump, vitreous in texture, and high in protein, but which when submitted to baking tests does not give entirely satisfactory loaves. This weakness has been attributed by Bailey (1925) to a lack of starch-splitting enzymes which produce available sugars for yeast fermentation. This enzymic action, which is responsible for the hydrolysis of starch to maltose, is defined as "diastatic activity," and the term will be used in this sense throughout this discussion, altho it is recognized that diastases have both liquefying and saccharifying power, the latter, according to Sherman and Schlesinger (1913), to a lesser degree in the case of certain diastases.

In addition to the rôle of diastase in determining the strength of flour, a number of other factors have been recognized as functioning in that connection. These include percentage of protein or gluten, ratio of gliadin to glutenin, "quality" of the gluten as indicated by water-imbibing capacity and in other ways, characteristics of starch, concentration of fermentable sugars, content and character of electrolytes, hydrogen-ion concentration, and buffer value of the flour and dough. The significance of these variables in their relation to flour strength has recently been discussed at length by Rumsey (1922), Sharp and Gortner (1923), and Bailey (1925), and need not be detailed here.

Sugars and Fermentation

The quantity of sugars in sound, normal flour is small. Glucose and maltose, both of which are directly assimilated by yeast, are present normally in flour to a limited extent, usually less than 0.15 per cent (Shutt 1907). Sucrose is present, often approximately one per cent. The total amount of disaccharids and monosaccharids, however, is insufficient food material for the yeast during fermentation of a dough. Consequently sugar must be added when a dough is mixed. While sucrose is used to greater extent than any other sugar, maltose and glucose are also utilized by the

baker. The percentage of sugar used varies widely, from 2 to 5 per cent according to a survey of experimental baking procedures made by Fitz (1924). A report of a large number of commercial dough formulas shows an average of 3.6 per cent sugar in sponge doughs, and 4.0 per cent in straight doughs.

Besides serving as carbohydrate food for the yeast, sugar contributes to flavor, sweetness, and a brown crust, properties which are desired by the American consumer of bread. The amount of sugar ordinarily added in the mixing of a dough is insufficient for these requirements, and it is neither practical nor economical to supply these needs with added sugar. Greater economy is practiced by allowing the diastatic enzymes to hydrolyze the starch of the flour. It is evident that the yeast must depend partly upon the carbohydrate food formed by enzymic hydrolysis, more particularly during the latter part of the fermentation period and the initial portion of the baking period.

The determination of the nature and chemical composition of the enzyme or group of enzymes known as diastase is a many-sided problem which has attracted many researchers. It has long been recognized that the action of diastase upon starch is not a single simple reaction. Starch is first converted into dextrins of varying molecular weights, which are then split up into molecules of maltose. The former reaction has been considered to be the result of hydrolysis by a liquefying enzyme, the latter by a saccharifying enzyme. That these two types of enzymes are present in wheat flour was demonstrated by Baker and Hulton (1908). Because of the two reactions, methods for determining diastatic activity have been founded upon two different principles. The liquefying power is measured with varying technic by the iodine method, which shows the amount of enzyme necessary to convert a given quantity of starch into compounds which do not give a blue color with iodine. The saccharifying power is the enzymic capacity for production of reducing sugar, which is measured by reduction of copper salts to cuprous oxid. In the former determination there is an excess of enzyme reacting upon a definite quantity of the substrate until the starch has been entirely hydrolyzed; in the latter the substrate is in excess, and the amount of the substrate which can be converted to reducing sugar in a given time is measured.

Much of the earlier work upon diastatic activity is conflicting and inconclusive, partly because of the variety of methods which

were employed for extracting the enzymes and determining their power. In the last decade and a half the work of Sherman and his co-workers has contributed substantially to our exact knowledge of amylases. In the first paper Sherman, Kendall, and Clark (1910) reviewed and criticized the methods which have been in use and proposed a new gravimetric method.

According to Kjeldahl's law of proportionality, the reducing sugar formed by action of a known amount of malt extract upon an excess of starch for a definite time is directly proportioned to the amount of actual amylase present, providing that no more than 40 per cent of the original starch is hydrolyzed. Sherman, Kendall, and Clark found that the rate of amyloclastic action was not constant up to the conversion of a considerable percentage of the original starch, but decreased. The importance of activating agents such as sodium chlorid or disodium phosphate was stressed by Kendall and Sherman (1910), since it was found that a purified enzyme preparation acting upon pure starch in pure water gave low activity. Apparently there was no regulation of hydrogen-ion concentration as the activating salts were added.

The amyloclastic and saccharogenic powers of malt and pancreatic amylases were compared by Sherman and Schlesinger (1913) who observed that in the case of pancreatic enzyme the amount of starch digested was about twice the amount of maltose produced, while malt enzyme formed maltose equivalent to two-thirds or more of the amount of starch digested. They suggest as a reason for this difference in starch-splitting and sugar-forming powers that the former expresses the amount of starch completely digested in a given time, while the latter signifies the maltose produced when the enzyme acts on an excess of starch. It is by no means certain that the same relationship between the two activities will hold in the case of a wheat flour dough, since, as mentioned above, the principle involved in making the two determinations is not the same. Concerning the importance of this relationship in wheat flour there is little evidence.

Determination of the nature of the end-products of hydrolysis by diastase (Sherman and Punnett, 1916) proved that glucose as well as maltose was produced. Malt diastase formed only a small amount of glucose. The authors were of the opinion that the glucose originated as a result of amylase action, believing that maltase was not present. The evidence of this conclusion regarding maltase, and the findings of Davis (1916) and Ling and

Nanji (1923) that maltase is present in barley do not harmonize, and it would be presumptuous to state which view is correct. It is worthy of note, however, that Collatz (1922) came to the conclusion that in panary fermentation of wheat flour doughs the amount of glucose formed is insignificant.

That starch as a substrate for enzymic action varies in its characteristics, was demonstrated by Sherman and Baker (1916). Their work showed that starch consists of two components, the more soluble, *B* amylose (or amylose) comprising 20 to 30 per cent, the less soluble, *δ* amylose (or amylopectin) comprising 70 to 80 per cent. When separated by centrifugal force, *δ* amylose was precipitated and appeared to consist of swollen grains. Action of three different amylases upon this more insoluble constituent produced less maltose than *B* amylose. It may be that the swollen starch granules of wheat which Whymper (1909) observed to resist digestion during germination were of the *δ* amylose type.

Sherman, Thomas, and Baldwin (1919) observed that the range of hydrogen-ion activity in which different amylases acted was, for malt pH 2.5 to 9.0, takadiastase pH 2.6 to 8.0, pancreatic pH 4.0 to 10.0, with optima at pH of 4.4 to 4.5, 4.8, and 7.0, respectively.

In the process of purification, Sherman and Neun (1919), and Sherman, Garard, and LaMer (1920) were able to separate amylase from protease only in the case of malt preparations; the best methods of purification of pancreatic amylase did not serve to eliminate proteolytic activity from the product.

It has been reported that amino acids have an influence upon enzyme activity. Sherman and Walker (1919) (1921) and Sherman and Caldwell (1921) (1922a) (1922b) investigated this, using several different amino acids. Glycine, alanine, phenylalanine, tyrosine, and aspartic acid favored enzymic hydrolysis; while histidine and tryptophane did not. Lysine had no effect upon liquefying power, but it did have upon saccharifying power. The favorable results were not due to changes in hydrogen-ion concentration as that was controlled, nor to combination with the products of the reaction. The authors are of the opinion that amino acids exert a protective action upon the enzyme. The deterioration of the enzyme is due in general to its hydrolysis, as it is protein in nature, and the addition of amino acids retards this hydrolysis.

The importance of diastatic activity in relation to baking strength has been recognized for many years. Wood (1907) stated that the size of the loaf depended more upon the diastatic capacity of the flour than upon the sugar content, and particularly upon the capacity for production of gas during the later stages of dough fermentation. Shutt (1907) concurred in this belief. Shortly after these papers appeared, Baker and Hulton (1908) and Ford and Guthrie (1908) reported the results of the first detailed studies of the enzymes of wheat flour. Baker and Hulton asserted that some of the carbon dioxid produced during the fermentation of a dough is formed from the maltose resulting from the action of diastase on the starch. They also demonstrated the presence of proteases in bread dough. The work of Humphries (1910), Swanson and Calvin (1913), Olson (1917), and others confirmed earlier statements that diastatic activity is an important factor in the fermentation of wheat flour doughs. Armstrong (1910) did not believe that diastatic activity was correlated with baking strength, because normal flours, in his estimation, contained an excess of enzymes. He admitted, however, that some flours were deficient in starch-splitting enzymes.

More recently Rumsey (1922), after working out a satisfactory method for determining the diastatic power of flour and studying the effects of several variables upon the determination, studied its relation to flour strength. Rumsey's method is a determination of the saccharogenic power of the diastase, measured by the reducing sugars found in a clarified extract from a flour-water suspension which had undergone autolytic digestion for one hour at 27° C. The diastatic activity is defined as the milligrams of maltose produced by the diastase in 10 grams of flour. Using this method, which will be described in detail later, Rumsey found that temperature exerted a marked influence upon the activity of wheat flour diastase, this activity increasing with the temperature to 63° C., above which there was a rapid decrease. Temperature, he stated, is the most important factor in the control of diastatic activity in the dough. At the usual fermentation temperature the rate of maltose production was found to be nearly constant between two and three hours digestion. The optimum hydrogen-ion concentration for the activity of wheat flour diastase was shown to be pH 4.7, with the optimum range pH 4.0 to 5.3. That this optimum range is barely reached by straight doughs, but is easily reached

by sponge doughs was demonstrated by the work of Bailey and Sherwood (1923).

Rumsey presented a series of curves which showed that the reducing sugar in a dough increased during the first hour of fermentation and then decreased when yeast was present; but when yeast was omitted there was a steady increase in reducing sugar, which was attributed to the hydrolysis of starch by diastases. From his study of fourteen flours of varying source and grade, the author concluded "the flour showing the greater diastatic power should show the greater baking strength and consequently the greater baking value, providing the relative quality and quantity of the gluten is the same."

Sufficient reference has been made to the literature upon diastatic activity to establish its importance as a factor among those which determine the bread-making capacity of flour. As wheat is grown under widely different conditions in the many wheat-producing areas of the world, it is to be expected that diastatic activity as well as protein content, vitreousness, weight per bushel, and other chemical and physical characteristics will vary. Less information is available, however, concerning the diastatic activity of flours from large numbers of wheat samples grown under different conditions. The results which Rumsey (1922) obtained with 9 flours of patent and straight grade from such widely separated sections as Kansas, North Dakota, Ohio, Montana, Utah, California, Alberta, and Saskatoon cover a range in diastatic power from 34 to 304, and are concrete evidence that there are wide variations in this flour characteristic.

In view of this fact it is logical that attempts should be made to supplement the diastatic power in certain cases at least. The agencies for increasing this power are limited. Products made from barley malt, namely malt extract and malt flour, have long been used by bakers, and are known to be of value. Altho there is no direct evidence to support the contention, it is highly probable that these preparations are used indiscriminately, regardless of the natural diastase content of the flour, with the result that in many cases the addition is unnecessary, while in others the amount added is insufficient. Another difficulty is attendant upon the use of malt extract in the bake shop of small output; the diastatic activity of the extract diminishes with age, when the container is opened and the material exposed as in ordinary usage, with the result that the baker must vary the percentage

used in order to contribute the same amount of diastase. This amount can not be estimated readily, as the average baker does not have the facilities for making tests of the extract to determine how much should be used to secure the best results. The enormous quantities of malt extract manufactured each year for use in the bakery have prompted investigation of the effects of the extract upon bread quality.

Collatz (1922) reported an extensive study of the effects upon flour strength of the addition of diastatic ferments and observed that "addition of malt flour and malt extract to doughs increased the volume of the resulting bread. In all cases the use of malt extract gave a superior loaf of bread in volume, grain, and texture, thus increasing the baking strength of the flour."

Two properties of active malt flours and malt extracts tend to reduce their usefulness in bread doughs. These are color and protease activity. Collatz and Racke (1925) have emphasized the hazards attendant upon the indiscriminate use of active or "diastatic" malt extracts in bread making. Their data indicate a progressive darkening of color, and diminution of texture score with increases in the "diastatic activity" of the malt syrup used in the dough batch. It is not yet evident, however, that the disadvantageous effects of active malt extracts can be wholly attributed to the amylases of the extract. We suspect that the active proteases may be responsible for certain of the undesirable effects that have been observed. It is highly desirable, therefore, that a source of amylolytic and saccharogenic enzymes be found which will contribute less to the impairment of crumb color and crumb texture in bread baking.

Wheat, as well as barley, increases in enzyme activity during germination, and is a logical selection as a source for diastatic enzymes. Wheat starch as a substrate for wheat diastase probably has no greater value than other starches, judging from the work of Sherman, Walker, and Caldwell (1919), who found that wheat, corn, rice, and potato starches prepared by the same method did not vary in the rate of reducing sugar production with several different enzyme preparations. Wheat diastase was not used, but the fact that amylases from pancreatin, saliva, barley malt, and *Aspergillus oryzae* gave the same results indicates that wheat amylase would not differ. Germinated wheat or the flour made from it is superior to barley malt flour as a medium for adding

diastase, as the admixture of wheat flour does not reduce the quantity of gluten.

Wheat containing germinated or sprouted kernels is found upon the market not infrequently, in fact, in some seasons the wheat shipped from certain localities very commonly contains sprouted kernels. The federal standards for wheat recognize the hazard in milling wheat of this character by allowing a maximum of 2.0 per cent of damaged kernels in No. 1 grade, 4 per cent in No. 2, 7 per cent in No. 3. While wheat containing sprouted kernels has been milled innumerable times, the milling trade in general considers it inferior in value to sound wheat. Recently there has been a tendency to regard with favor wheat of this nature for blending purposes, and in a few cases which have come to our attention a premium has been paid instead of a discount being demanded for wheat containing small percentages of sprouted kernels. The fact must not be overlooked, however, that wheat which has sprouted while in shocks in the field contains kernels in varying stages of germination, the more advanced stages capable of exerting such a damaging influence that the miller can not be certain of the character of the flour that the wheat will yield.

There have been a number of investigations of the effects of sprouted wheat upon milling and baking quality. These have been reviewed by Bailey (1925).

While most of the reports in the literature dealing with germinated wheat cover experiments made to determine the changes undergone and the effects upon milling and baking, there is record of a patent issued to Dombach (1912) for a process of "treating grain with like grain previously germinated slightly." The process is claimed to produce a high-grade flour from low-grade grain. The grain to be germinated is softened by liquid for 6 to 10 hours, the liquid is removed and the swelling and germination are allowed to take place, with a stream of air forced through the mass by pressure. When the process is completed the germinated grain is mixed with normal grain and the whole mass is brushed, polished, and ground. Special apparatus is described for carrying out these operations. It is mentioned that it is necessary, especially when the grain in question is wheat, that the swelling or germinating process should be rapidly carried out, as wheat easily and rapidly passes over into fermentation.

Summarizing the information contained in the various papers dealing with sprouted wheat, it seems safe to conclude that ger-

minated wheat is a source of enzymes which can be utilized for supplementing the diastatic activity in wheat; that wheat during germination and subsequent scouring in preparation for milling suffers a loss of dry matter, due in part to increased respiration, in part to translocation of food materials to the plumule and rootlets, and in part to dislodging of the embryo; that the addition of sprouted wheat to the mill mix will result in a milling loss in direct proportion to the amount added, but not equal to it; that small percentages of sprouted wheat effect improvement in the flour produced from wheat normally low in diastatic activity, but that large percentages cause impairment of flour quality; that the character of the sprouted wheat, as influenced by the time, temperature, and other conditions under which germination took place has an important bearing upon the quality of the flour. In most of the reported investigations no attention was paid to diastatic activity; in a few, diastase was estimated by the iodine method, which is a measure of the amyloclastic power, and does not give an exact indication of the amount of carbohydrate food available for the yeast during the fermentation of a dough.

Experimental

The problem.—In view of the many facts discussed thus far, the problem of this study was chosen. The object of the investigation was to supplement the diastases naturally occurring in the wheat by the addition, in the milling process, of wheat which had been allowed to germinate for a short time under careful control. By this means an effort was made to regulate the diastatic activity in the flour, and improve, if possible, the baking quality of flour milled from wheat low in diastases by raising the diastatic activity to a higher level. By making the addition during the milling process and varying the amount added according to the diastatic power of the wheat, it should be possible to turn out a finished product satisfactory in its diastase content, thus relieving the baker of the necessity of adding diastatic preparations.

The experimental work may be divided logically into two parts. Before attempting to mill, on a large scale, wheat mixtures prepared as indicated, it was deemed advisable to make a preliminary study of flours milled on a small scale. Accordingly, a program was outlined which included the milling of 2000-gram portions of wheat contain-different percentages of germinated wheat. Using the results of these trials as a basis for tests with larger quantities, tests with 50-bushel lots were later made on a commercial scale.

Germination of wheat.—The germination of the wheat was carried on in a specially constructed germinator, and as this was used for all the germinating it will be described somewhat in detail. A galvanized tank was secured having an outlet in the hoppered bottom controlled by a valve. A dozen trays were constructed by stretching burlap over a square wooden frame 1¾ inches deep. On the bottom of each tray were nailed cleats which separated the trays, when stacked, by a 2½ inch space. Six trays could be placed in the tank at one time. The wheat to be germinated was spread upon the trays, which were stacked in the tank, and the tank was filled with water. After 4 to 5 hours had elapsed, to allow imbibition of sufficient water for germination, the excess water was drawn off through the outlet. Evaporation from the wheat was retarded by raising the humidity of the atmosphere in the tank. To accomplish this, two inches of water was kept in the bottom of the tank and wet burlap was thrown over the top. The temperature was maintained at approximately 16-18° C. The relationship of temperature to rate of germination was followed by Reynolds (1904), who observed that in wheat incubated at 55-65° F. (13-18° C.) the radicle first appeared in 3 days, while that held at 68-72° F. (20-22° C.) came to the same stage in 2 days.

As the carbon dioxide of respiration, if permitted to accumulate, would affect the rate of germination, it was removed by attaching the outlet pipe of the tank to an aspirator which was operated continuously. Occasional sprinkling and stirring kept the wheat moist and promoted uniform germination.

Red Fife wheat grown at University Farm, St. Paul, was used in the first part of the study. Ten pounds of the cleaned wheat was allowed to germinate as just described, one-half for 3 days, the other half for 5 days. After removal from the germinator, the grain was dried as rapidly as possible in a warm room with a liberal circulation of air provided by electric fans. In less than an hour the sprouts and roots were shriveled, and in 5 or 6 hours the grain was dried sufficiently to store safely. The remainder of the ungerminated wheat and the two portions of germinated wheat were scoured separately in a Eureka scourer, which is designed to remove chaff, dirt, and some of the hairs from the blossom ends of the kernels. In passing through the scourer the germinated wheat lost a portion of the germ, and was separated from the dried sprouts and roots. In cleaning there was naturally somewhat greater loss in the germinated wheat, but the greater part of this loss was material which in the

process of milling would be contributed to the feeds rather than to the flour.

As scouring removed the sprouts and germ, the total protein in the germinated and scoured wheat was somewhat lower than in the normal or ungerminated grain. The difference after 5 days sprouting, was about 0.5 per cent.

Catalase activity of wheat—It has been shown that there is a correlation between catalase activity and some other cell activities, and as this determination can be made easily and rapidly, it was thought that it might serve as an index to the extent of germination. Catalase activity was determined in the ground wheat meal, both ungerminated and germinated, by the volumetric method used by Bailey (1917), modified to conform with the principle advocated by Osterhout (1918) that biological reactions should be measured in terms of the time required to produce a given change rather than the amount of change observed in a given time. Following this principle, the method involved the measurement of the time required to liberate 10 cc. of oxygen from a suspension of wheat meal to which H_2O_2 had been added. The wide-mouthed bottle containing the wheat meal suspension was mounted on a shaker, a miniature wagon attached to an eccentric which was operated by a motor. The agitating device was operated continuously while the reaction was in progress at a speed just rapid enough to keep the wheat meal in suspension. One gram of wheat meal was placed in the bottle, suspended in 100 cc. of water at 20° C. and there was then added 4 cc. of neutralized H_2O_2 capable of liberating 20 cc. of oxygen with excess catalase. The time required to collect 10 cc. of oxygen was measured. The results obtained with Fife wheat are reported in Table I. It is readily apparent from this that there is a regular increase in catalase activity during at least the first five days of germination. This is in agreement with the findings of Choate (1921). It is suggested that this determination may prove a useful index of the extent to which germination has taken place in wheat containing a small percentage of sprouted kernels.

TABLE I

CATALASE ACTIVITY OF RED FIFE WHEAT GERMINATED AND UNGERMINATED

Treatment of wheat	Weight of sample	Time to liberate 10 cc. of oxygen	$1/t \times 10^2$
	Gram	Min.	
Ungerminated	1	29.00	3.45
Germinated 3 days	1	11.25	8.89
Germinated 5 days	1	7.25	13.80

In order to determine the effect of germinated wheat in varying amounts upon the diastatic activity of flour, ten lots of wheat were

prepared. Two of these consisted entirely of ungerminated wheat to serve as controls; four contained 5, 10, 20, and 40 per cent, respectively, of the wheat germinated 3 days in the manner described; four contained the same percentages of wheat germinated 5 days. Moisture determinations were made upon each lot, and a 2000-gram portion, after tempering to 15 per cent moisture, was milled on a three-stand Allis-Chalmers experimental flour mill. Yields of the feeds and the flour were determined, and the patent flour representing 75 per cent of the total flour was saved for subsequent study.

Milling tests.—The results of the milling tests are shown in Table II. The total flour obtained varies considerably with the different samples, and while the variation is not uniform, since there is a large experimental error in milling tests of this kind, the tendency is toward a lower yield of flour when a large proportion of germinated wheat is added. This confirms the experience of other investigators.

TABLE II

MILLING TESTS OF FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Lab. No.	Germinated wheat	Total flour	Shorts	Bran	Total feed	Total products
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
			Germinated 3 days			
501	0	74.4	8.9	15.4	24.3	98.7
503	5	72.4	9.2	18.3	27.5	99.9
504	10	73.7	10.1	15.4	25.5	99.2
505	20	73.0	10.2	16.0	26.2	99.2
506	40	73.4	11.4	12.4	23.8	97.2
			Germinated 5 days			
502	0	74.1	7.0	17.2	24.2	98.3
507	5	74.2	8.8	15.6	24.4	98.6
508	10	72.8	6.9	17.7	24.6	97.4
509	20	72.0	8.0	17.7	25.7	97.7
510	40	70.9	7.3	18.6	25.9	96.8

Diastatic activity determinations.—As the prime object of this investigation was the control of diastatic activity, this portion of the work will be discussed first. The method of Rumsey, with slight modifications which did not effect any change in the fundamental principles, was used for all determinations of diastatic activity. This method will be described in detail, for strict adherence to the recommendations of Rumsey determine largely the accuracy of the results. The method involves digestion of a flour-water suspension, clarification of the extract, and determination of its reducing sugar content. A 10-gram sample of flour was weighed out, transferred to a 200-cc. Kohlrausch flask, and brought to 27° C. in a water bath; 100 cc. of water (27° C.) was added, and the flour put in suspension by rotating the flask, after which it was loosely stoppered and placed in the bath

for 60 minutes, with gentle shaking at 15-minute intervals to keep the flour in suspension. At the end of the digestion period, the suspension was diluted to approximately 175 cc. and clarified by adding 3 cc. of 15 per cent sodium tungstate ($Na_2WO_4.2H_2O$) solution, acidifying with 0.8 cc. H_2SO_4 (concentrated diluted 1:1) to a pH of about 2.0, indicated by the pink color produced with 5 or 6 drops of 0.04 per cent aqueous thymol blue (Clark, 1922) solution. This clarification stopped enzymic action immediately and precipitated the protein. After dilution to the 200-cc. mark, the liquid was centrifuged, and a 50-cc. portion of the clear supernatant liquid was removed for the reducing sugar determination.

A blank determination was made upon the same flour to correct for the original reducing sugar content. This was made in a similar manner except that the clarifying reagents were added immediately after the flour was put in suspension, thus halting enzymic action and allowing the estimation of natural reducing sugar in the flour.

Rumsey has discussed thoroly the reasons for the procedure which he adopted. It will suffice to remark here that this method for determining diastatic activity approaches more nearly the actual conditions under which the diastase operates in dough fermentation than any other proposed method. Of the several variables which affect the determination and must be controlled, time, temperature, and concentration of flour are easily regulated by the operator. Hydrogen-ion concentration is determined by the flour, and in normal flours is considerably lower than the optimum for diastatic hydrolysis. Rumsey found that the pH of the different patent flours with which he worked varied from 5.7 to 6.1. Reference to the graph in which he shows the effect of changing the hydrogen-ion concentration, indicates that this variance of 0.4 pH may have had some influence upon the amount of maltose produced, and that adjustment of the pH to the same level in each determination might have changed the relative diastatic power of the series of flours.

Sorenson (1924) commented on Rumsey's work, and criticized his method of estimating diastatic activity for its lack of hydrogen-ion control. Comparing two of Rumsey's flours of the same diastatic power, Sorenson stated that the one with the pH 0.6 higher must have had considerably more power to produce an equivalent quantity of maltose. He suggested determining the buffer value of flour before determining the diastatic activity, thus being in a position to regulate the hydrogen-ion concentration of the flour-water suspension. By using three different concentrations, the method used at the Carlsberg

laboratories, a curve could be constructed and flours compared at a definite pH.

The criticisms of Sorenson are based upon sound reasoning, and a comparison of diastatic power at a uniform hydrogen-ion concentration approaching the optimum is desirable from the standpoint of absolute accuracy. In justification of the method of Rumsey, however, it should be stated that from the practical standpoint the consumer of flour is concerned about what will occur when that flour is made into a dough and baked. Adjustment of the pH to the optimum during the determination of diastatic activity will give a result which may bear no relation to the activity of the enzyme in a bread dough, since bread doughs rarely reach that optimum in their fermentation. The hydrogen-ion concentration and the buffer value of the natural flour influence greatly the result of the diastatic determination, but the same influence is exerted in a bread dough, and a method of estimating enzymic activity which simulates the conditions of dough fermentation will yield the most useful results. Until scientific control of bakery practices advances to the stage that the pH of commercial doughs is regulated accurately, regardless of the pH of the flour used, determinations which will aid in evaluating the flour as it exists will continue to be extremely useful.

Olsen and Fine (1924) have recently contributed an excellent paper which adds considerable to our knowledge of the effects of variables upon diastatic activity. The work of Sherman, Thomas, and Baldwin (1919) showed that the optimum pH for purified malt diastase is 4.5; Collatz (1922) found for the same enzyme in malt extract 4.26; Rumsey (1922) found 4.7 for wheat diastase; the data of Gore (1925) showed an optimum range of pH 4.5 to 5.5, with the highest value at 5.07 for malt diastase. Gore used a polarimetric modification of Lintner's method for estimating the reducing sugar, digested his preparations at 21° C., and controlled the acidity by use of a buffer solution containing acetic acid and sodium acetate. Olsen and Fine stated that the apparent lack of agreement in optimum H-ion concentration is not subject to criticism, but indicated that temperature is an important factor, as different temperatures were used by the different investigators. It was demonstrated that temperature exerts a great influence upon the optimum hydrogen-ion concentration for the activity of malt diastase. They found experimentally the following optima, in terms of pH; 4.3 at 25° C., 5.1 at 45° C., 5.7 at 60° C., 6.05 at 69° C. On constructing a curve from these data they concluded that "the optimum pH appears to be a linear function of the tem-

perature." An equation was derived for calculating the optimum at any temperature:

$$p = \frac{85.5 + t}{25}$$

where p is the optimum pH at temperature t. Several values calculated from this equation coincided with the curve drawn from experimental data. It was suggested that the higher pH at higher temperatures might be explained by greater activity of the hydrogen ions present. They also pointed out that the apparent difference between the optimum for liquefaction and that for saccharification is due to the temperatures at which the determinations were made.

Methods for estimating reducing sugars in a water extract are numerous, and several reliable ones are at the disposal of the researcher. The method of Quisumbing and Thomas (1921) was chosen because in their careful study they have seemingly brought under control all the variables which affect the reduction of Fehling solution. The alkaline tartrate solution contains 65 grams sodium hydroxide and 175 grams Rochelle salts per 500 cc; the copper solution contains 41.2 grams $CuSO_4$, 5 H_2O per 500 cc.

The reducing sugars in the diastatic determinations were measured by the following procedure:

After the clarification of the flour suspension, a 50-cc. aliquot in a 400-cc. beaker, representing 2.5 grams of flour, was neutralized with sodium hydroxide solution and the copper sulfate and alkaline tartrate solutions were added, 25 cc. of each. The beaker, covered with a water glass, was then placed in a water bath maintained at 80° C., for 30 minutes, after which it was removed and the liquid filtered rapidly with suction through a tared Gooch crucible prepared with an asbestos mat. The cuprous oxide was washed with hot water, alcohol, and ether, dried and weighed. The final determination of the cuprous oxide by direct weighing instead of by electrolytic precipitation of metallic copper as recommended by Quisumbing and Thomas has a larger experimental error, but for estimating the result of a biological reaction such as the one with which we are concerned here, it is entirely satisfactory. From the weight of the oxide the weight of the anhydrous maltose was obtained by reference to the tables prepared by Quisumbing and Thomas. The maltose found in the digested flour suspension, less the maltose in the blank, gave the maltose produced by enzymic action. Diastatic activity in Rumsey's units is the milligrams of maltose produced by autolytic digestion of 10 grams of flour at 27° C. for one hour.

The results of the determinations of diastatic activity of the flours milled from mixtures of germinated and ungerminated wheat are given in Table III. Figure 1 shows the same results expressed graphically. Here is conclusive evidence that substantial increases in diastatic power can be produced by incorporating germinated wheat in the mill mix. It will be noted that the addition of 5 per cent of the 3-day germination doubled the enzymic activity, 10 per cent nearly trebled it, but that larger percentages did not result in the same relative increase.

TABLE III

Diastatic Activity of Flours Milled from Fife Wheat with and Without Admixture of Germinated Wheat

Flour No.	Germinated wheat	Original maltose per 10 gm. flour	Diastatic activity, maltose produced by 10 gm. flour
	Per ct.	Mgm.	Mgm.
		Germinated 3 days	
Av. 501 and 502	0	22.4	86.4
503	5	41.6	173.2
504	10	37.2	222.4
505	20	45.6	233.6
506	40	120.4	278.8
		Germinated 5 days	
507	5	76.8	237.6
508	10	102.8	266.4
509	20	137.6	327.2
510	40	272.4	385.6

In the case of the 5-day germination, the rate of increase in diastatic activity begins to diminish after 5 per cent is exceeded. Collatz (1922) found the same to be the case with most of the flours to which he added malt extract or malt flour. Rumsey (1922) found that with the flours he studied, those with values in the neighborhood of 250 scored the highest in baking value. A small per cent of germinated wheat was capable of raising the flour in diastatic power to within the range which appears to be desirable. The wheat germinated for 5 days had a much greater influence upon the production of maltose, but further tests of these flours, described later, show that this period of germination was too long.

Reducing sugar originally in the flour is shown in both the tables and the figures. The longer period of germination is responsible for a much larger quantity of sugar in the flour, as well as increased production of sugar.

Changes in protein during germination.—During germination of wheat, many changes take place in the kernel other than those directly related to diastatic activity. Changes in the proteins, especially the gluten proteins, have a particular significance because of the relation-

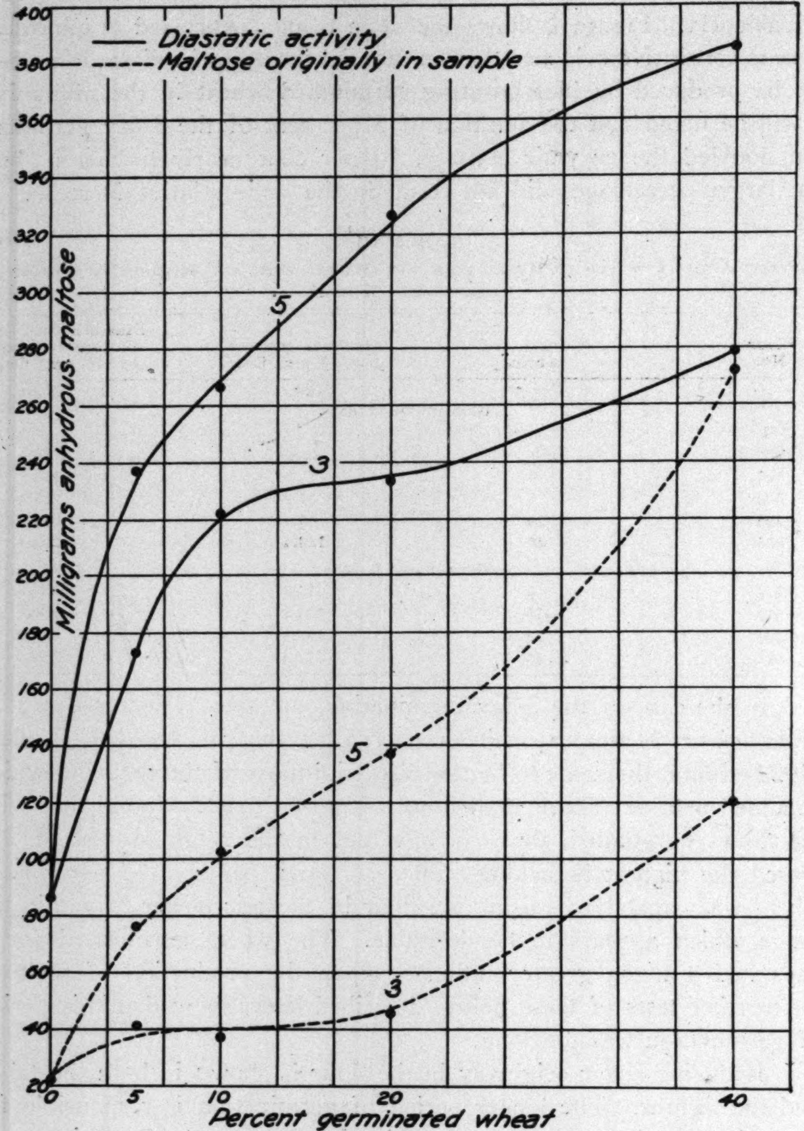

Fig. 1. Diastatic Activity and Original Maltose in Two Series of Flours Varying Percentages of Wheat Germinated 3 Days and 5 Days

ship between both quality and quantity of protein and the baking capacity of flour. The literature contains conclusive evidence that protein-splitting enzymes are more active in wheat which has been allowed to germinate. Balland (1884) found that germinated wheat had the power of liquefying gluten, and considered that the origin of the ferment was adjacent to the embryo. Abderhalden and Schittenhelm (1906) demonstrated that the juice pressed from germinating seeds of wheat was capable of hydrolyzing polypeptides. The work of Vines (1906, 1909), an extensive study of proteases in seeds and other plant tissues, established the presence of proteases in both germinated and ungerminated seeds. Two kinds of enzymes appeared to be active, one peptic in nature, the other ereptic. The ungerminated seeds contained an enzyme which caused hydrolysis of Witte-peptone and acted very slowly upon the reserve protein in the seeds; the germinated seeds elaborated an enzyme capable of digesting fibrin, the activity varying with different seeds. Bialosuknia (1909) also reported that plant proteases active upon plant proteins were present in all seeds germinated from 2 to 5 days.

As the germination of wheat is responsible for increased activity of proteases as well as diastases, some attention must be paid to the effects of the former upon the quality of flour milled from wheat containing a small percentage of germinated kernels. Ford and Guthrie (1908) observed that the addition of a small amount of protease to flour would seriously affect its quality, reducing the tenacity of the gluten and hence the gas-retaining power. They suggested that proteases might be responsible for the unsatisfactory results obtained by the use of some malt preparations. Their findings were in harmony with those of Baker and Hulton (1908), who recognized the deleterious effects of protease action but were unable at that time to demonstrate the presence of proteoclastic enzymes in wheat flour, and attributed the hydrolysis of protein to the enzymes in the yeast. Weaver and Wood (1920) found that traces of pepsin and trypsin added to a dough impaired its bread-making properties.

Large amounts of nitrogen in amino form were considered by Swanson and Tague (1917) to be an indication of certain undesirable qualities in flour. Sorenson's formol titration method of estimating the amount of titratable nitrogen was used as a measure of a certain degree of protein hydrolysis, and it was found that wheat would show increased amounts of titratable nitrogen if exposed to unfavorable conditions so that proteolytic enzymes could attack the protein.

Stockham (1920) demonstrated the presence of proteases in flours of different grades by the liquefaction of gelatin. Flours from sprouted wheats showed higher activity, and less deterioration of protease with age. Both flour and feeds milled from the germ-end half of the kernels liquefied gelatin quite rapidly, while the same products from the blossom end of the kernels liquefied gelatin much more slowly. Sharp and Elmer (1924) concluded from their studies of the proteolytic activity of flours milled from wheats harvested at various stages in their growth that "the proteases of wheat flour are capable of digesting the flour if given sufficient time in which to act."

Protein cleavage studies.—In wheat flour there are always present nitrogenous compounds which are soluble in water. Part of these are protein in nature, part are non-protein and vary in their complexity, including peptids and simple amino compounds. The amounts of these several soluble constituents vary somewhat in different flours. Proteoclastic activity is responsible for hydrolytic changes which will increase the proportion of simpler nitrogenous compounds, and these can be fractionated by the use of certain reagents which are rather specific in their action upon nitrogen complexes. Determination of the nitrogen in the different fractions offers a means of estimating the extent of hydrolysis.

Reagents which can be used for the purpose of fractionating the soluble nitrogen are stannous chlorid solution and copper hydroxid. Scherning (1897) was probably the first to use the former, while the latter was used early by several investigators including Ritthausen (1872), and Stutzer (1881), who described the preparation and use of the freshly precipitated copper hydroxid sludge called Stutzer's reagent. Osborne and Leavenworth (1916) and also Blish (1918) modified the use of this reagent, the latter showing by his work that it precipitated protein and its products of hydrolysis more complex than peptids and amino compounds. Both the tin and the copper reagents were used by Olsen and Bailey (1925), who improved the technic of their use by controlling the hydrogen-ion concentration of the liquid to which they had been added.

To determine whether the addition of germinated wheat would cause an increase in the simpler nitrogeneous compounds in the specially prepared flours, determinations of nitrogen were made following essentially the fractional precipitation method of Olsen and Bailey. A 20-gram sample of flour was suspended in toluene water, diluted to 200 cc., and digested with occasional shaking for 1 hour in a water bath, after which the suspension was centrifuged, and 3 50-cc. portions

of the supernatant liquid were removed. The first was used for the determination of the total soluble nitrogen; to the second in a 100-cc. volumetric flask was added 5 cc. stannous chlorid reagent and sufficient 5 per cent sodium hydroxid solution to bring a faint blue with bromcresol purple (pH approximately 5.9), after which dilution to 100 cc. was made and 80 cc. was removed for nitrogen determination. To the third in a similar volumetric flask was added 20 cc. 0.2 N sodium hydroxide, phenolphthalein indicator solution, and sufficient 0.2 N. copper sulfate to cause a change from blue to green, followed by dilution to 100 cc. and removal of 80 cc. for determination of nitrogen. Nitrogen was determined by the Gunning modification of the Kjeldahl method. The nitrogen found in the three extracts is reported as total water-soluble, Sn-non-precipitable, and Cu-non-precipitable, respectively.

TABLE IV

Total Nitrogen and Nitrogen in Various Fractions in Flours Milled from Wheat With and Without Admixture of Germinated Wheat

Flour Lab. No.	Germinated wheat	Total nitrogen	Water-soluble nitrogen	Sn-non-precipitable nitrogen	Cu-non-precipitable nitrogen
	Per cent	Per cent	Per cent	Per cent	Per cent
		Germinated 3 days			
Av. 501 and 502	0	2.091	0.264	0.058	0.020
503	5	2.063	0.267	0.046	0.025
504	10	2.050	0.274	0.069	0.032
505	20	1.993	0.284	0.088	0.032
506	40	2.037	0.315	0.094	0.038
		Germinated 5 days			
507	5	2.017	0.277	0.081	0.029
508	10	1.979	0.285	0.092	0.025
509	20	1.981	0.304	.....	0.031
510	40	1.965	0.404	0.163	0.053

Determinations of total nitrogen in the flours were made at the same time. These results are reported in Table IV. It will be noted that there was a slight decrease in the total nitrogen, and an increase in the fractional nitrogen, more marked in the case of the flours from mixtures of wheat germinated 5 days. Because of the difference in total nitrogen in the flours, the quantities of soluble, Sn-non-precipitable, and Cu-non-precipitable nitrogen have been calculated as per cent of the total, and are expressed as such in Table V. The same results are recorded graphically in Figure 2. The changes in soluble nitrogen are considerable and probably are not due entirely to change in the chemical composition of the protein. The work of Johnson and Bailey (1924) completed since the flours mentioned were studied, established that the quantity of protein nitrogen remaining in the liquid after centrifuging a water suspension of a flour or dough was deter-

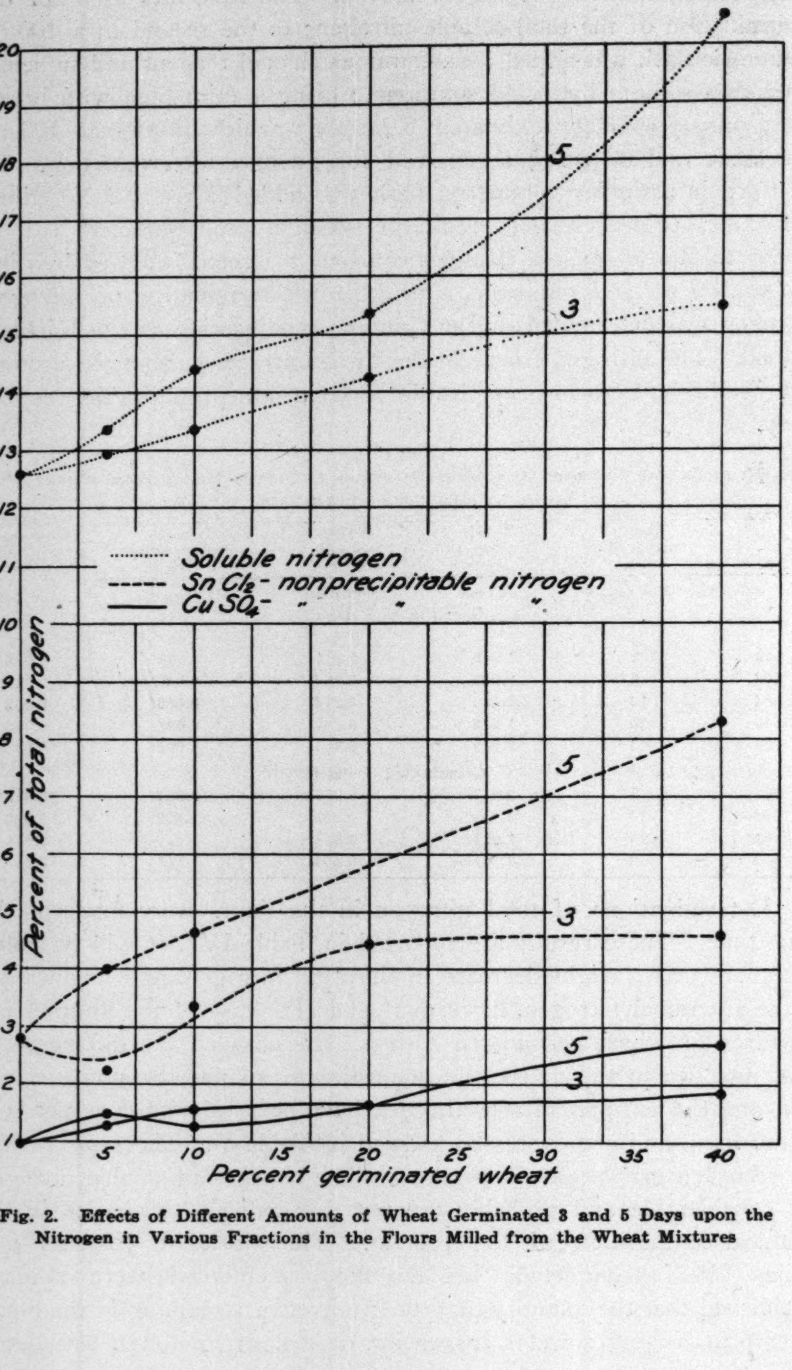

Fig. 2. Effects of Different Amounts of Wheat Germinated 3 and 5 Days upon the Nitrogen in Various Fractions in the Flours Milled from the Wheat Mixtures

mined almost entirely by the hydrogen-ion concentration of the suspension. With the increasing hydrogen-ion concentration (within the limits of the experiment) increasing proportions of the flour protein were dispersed in a form which could not be separated by centrifuging.

TABLE V

NITROGEN IN VARIOUS FRACTIONS, CALCULATED AS PER CENT OF TOTAL NITROGEN IN FLOURS MILLED FROM WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

		In terms of total nitrogen		
Flour Lab. No.	Germinated wheat	Water-soluble nitrogen	Sn-non-precipitable nitrogen	Cu-non-precipitable nitrogen
	Per cent	Per cent	Per cent	Per cent
		Germinated 3 days		
Av. 501 and 502	0	12.60	2.80	0.98
503	5	12.94	2.23	1.21
504	10	13.37	3.37	1.56
505	20	14.25	4.42	1.61
506	40	15.46	4.62	1.87
		Germinated 5 days		
507	5	13.73	4.02	1.44
508	10	14.40	4.65	1.26
509	20	15.35	....	1.56
510	40	20.56	8.30	2.70

The flours used in this study were not uniform in hydrogen-ion concentration, and in view of this fact the changes in water-soluble nitrogen have less significance than the changes in nitrogen not precipitated by tin and copper, where the original acidity did not have the same influence. Some difficulty was experienced in obtaining clear extracts with the tin reagent, but none with the copper solution. Comparison of the curves for the 3-day and 5-day germinations shows that considerably more of the products of protein decomposition were contributed by the latter. This would suggest that the gas-retaining powers of the gluten suffered by the admixture of wheat germinated for the longer period. Evidence which follows later supports this contention.

An effort was made to employ changes in viscosity of the acidulated flour suspension as a measure of the extent of proteolysis in the sprouted wheat flour. Such differences in viscosity as were made by the method employed were within the experimental error of the method. A more refined procedure was followed in later studies which will be detailed in another section.

Baking tests.—Flours milled from wheat mixtures containing germinated kernels have exhibited a greater diastatic activity than flours milled from ungerminated wheat. Nitrogen determinations have indicated that undesirable changes in the proteins may have taken place. While such chemical determinations are extremely valuable, and can

be made with a high degree of accuracy, we must still rely upon the baking test of flour to demonstrate in a practical manner how the flour will behave during fermentation with yeast and when subjected to the heat of the bake oven. Baking tests were made with the flours, therefore, using essentially the method described by Bailey (1916). The formula for the dough was as follows:

	Grams	Per cent
Flour	450.00	100.00
Yeast	11.25	2.5
Sugar	11.25	2.5
Salt	6.75	1.5

The doughs were mixed in a mechanical dough mixer for 5 minutes, placed in a fermentation cabinet at 28° C., transferred to a proofing cabinet at 34° C. when two-thirds of the dough representing 300 grams of flour was panned, and baked when ready for the oven.

In order to afford comparisons under the same conditions, the samples of the 3-day series were baked one day and the 5-day series the following day. The results are given in Table VI.

TABLE VI

BAKING TESTS OF FLOURS MILLED FROM FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	Absorption	Fermentation time: Total	Fermentation time: Proofing	Loaf volume	Texture	Color	Expansimeter
	Per cent	Per cent	Min.	Min.	cc.	Score	Score	cc.
			Germinated 3 days					
501	0	58.8	250	67	1980	99	100	1100
503	5	58.8	245	62	2030	100	100	1050
504	10	58.2	235	60	2080	99	100	1260
505	20	58.2	235	71	2350	101	100	1200
506	40	58.6	230	64	2400	98	100	1020
			Germinated 5 days					
502	0	58.8	260	60	2250	100	100	1010
507	5	58.6	255	55	2350	98	100	1200
508	10	58.0	245	53	2400	95	100	1300
509	20	58.0	255	55	2500	96	100	1250
510	40	58.0	230	47	2350	94	100	1050

Absorption diminished slightly with increase in germinated wheat, altho there was no regular decrease. The fermentation time was shortened appreciably as the amount of germinated wheat was increased to the extent of 20 minutes in the first series and 30 minutes in the second, when the extremes are compared. The majority of this saving in time was in the proofing period, where the higher temperature is conducive to more rapid enzymic action and, therefore, a faster raising of the dough. This is more noticeable in the 5-day series, where the addition of 40 per cent shortened the proofing time nearly one-fourth.

Loaf volumes and expansimeter volumes were increased by the addition of germinated wheat, until the amount added reached 20 per cent of wheat germinated 5 days. The 3-day series showed a fairly regular increase in loaf volume. The 5 per cent mixture gave an excellent loaf of bread, the 10 per cent a larger volume but slightly poorer texture, while the 20 per cent exceeded both of them in texture and volume. The 5-day germination series gave the largest volume, but in all cases the grain and texture suffered. Even the 5 per cent mixture showed somewhat poorer grain and texture than the control, and the others were progressively poorer. None of these loaves was satisfactory and all shrunk upon cooling. Comparison of No. 507 and No. 505 shows that 5 per cent of 5-day germination gave the same loaf volume and diastatic activity as 20 per cent of 3-day germination, but that the former yielded bread with poorer grain and texture, due probably to increased protease activity, resulting in impairment of gas-retaining properties of the gluten. This demonstrated the importance of regulating the length of time which the wheat germinated.

The external color of the loaves was good, a rich brown, somewhat darker with the larger amounts of germinated wheat, indicating that higher diastatic activity, particularly during the proofing period, increased the quantity of sugars which caramelized in the heat of the oven.

It should be mentioned here that it was not anticipated that good baking flours would be produced from wheat containing large percentages of germinated kernels. These larger amounts were used because study of the changes taking place was facilitated when the magnitude of these changes was great.

Determination of hydrogen-ion concentration.—The importance of the hydrogen-ion concentration of flour has been mentioned earlier in this discussion. This determination was made upon the flours milled from germinated wheat mixtures by the conventional electrometric method. The water extract for charging the electrode was prepared by extracting 10 grams of flour with 50 cc. carbon dioxid-free distilled water in a water bath at 25° C. for 1 hour, after which the suspension was centrifuged and the supernatant liquid removed.

Determination of titratable acidity.—Titratable acidity was also determined, by digestion of 18 grams of flour in 200 cc. of carbon dioxid-free distilled water for 1 hour, and titration of a filtered aliquot with 0.01 N. sodium hydroxid using phenolphthalein indicator. The acidity determinations are given in Table VII.

TABLE VII

HYDROGEN-ION CONCENTRATION IN TERMS OF pH, AND TITRATABLE ACIDITY AS LACTIC ACID OF FLOURS MILLED FROM FIFE WHEAT WITH AND WITHOUT ADMIXTURE OF GERMINATED WHEAT

Flour Lab. No.	Germinated wheat	H-ion concentration as pH	Titratable acidity as lactic acid
	Per cent		Per cent
		Germinated 3 days	
501	0	5.99	0.19
503	5	5.98	0.19
504	10	6.01	0.22
505	20	5.92	0.21
506	40	5.89	0.23
		Germinated 5 days	
502	0	6.03	0.21
507	5	5.84	0.20
508	10	5.82	0.20
509	20	5.79	0.21
510	40	5.71	0.23

Inspection of this table shows that there is a significant increase in hydrogen-ion concentration with increasing amounts of germinated wheat. The effect of wheat germinated 5 days is more pronounced. The influence of germinated wheat upon the titratable acidity of the flour is slight, but sufficient to be recognized.

Summary of preliminary experiments.—Summarizing briefly at this point the results of experiments with flours milled from mixtures of normal and germinated wheat, it has been shown that diastatic activity can be increased by the addition of germinated wheat; in fact, can be carried beyond the range which present knowledge indicates is satisfactory from the baking standpoint. The length of the germinating period was found to be important. A small quantity of wheat germinated for 5 days was much less desirable than two or four times that quantity of wheat germinated 3 days. Large quantities of germinated wheat reduced the baking value, probably very largely because of the activity of proteases which were unavoidably contributed with the diastases, or to the presence of a relatively large proportion of partially hydrolyzed gluten. There is evidence that protease activity is not so marked in wheat germinated for the shorter period.

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